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BIOLOGICAL BULLETIN

ARE PARTICULAR CHROMOSOMES SEX DETERMINANTS?

THOS. H. MONTGOMERY, JR.

The past decade has witnessed greatly renewed interest in the problems of sex determination, due very largely to the study of hybridization and the broad application of the results. Concurrently the investigation of the germ cells has increased in amount in a geometrical ratio; never before have these cells so fully engaged the thoughts of biologists, and our knowledge of the complex chromosomal activities has increased to an extent unpredicted.

In particular have multiplied investigations of the modified chromosomes, now described in animals for the spermatogenesis in insects, araneads, myriopods, copepoda, Sagitta and birds: and for the oogenesis in echinoderms, the cat, and possibly some insects (*Pyrrhocoris*, *Gryllus*). Following the terminology given by me in 1906, these may be collectively named allosomes, a term more convenient than my earlier one of heterochromosomes, in contrast to the unmodified chromosomes or autosomes. They have received a great variety of names: accessory, special, lagging, heterotropic, sex chromosomes; idiochromosomes, microchromosomes, diplosomes, gonochromosomes, chromatin nucleoli, etc. Any body within a nucleus that stains like chromatin should not, however, be considered an allosome until its chromosomal nature be ascertained.

Within the past eight years an hypothesis has arisen ascribing sex-determining properties to these allosomes, and my object is to treat this hypothesis first historically, and second critically.

A. The Hypothesis.

The first statement of the hypothesis is due to McClung in 1902, after investigation of the unpaired accessory chromosome in the spermatogenesis of Orthoptera — that kind of modified chromosome named by me in 1906 the monosome. McClung's conception of the function exercised by the accessory chromosome is "that it is the bearer of those qualities which pertain to the male organism, primary among which is the faculty of producing sex cells that have the form of spermatozoa." He recognized also that there must be selective fertilization, that to the ovum "come the two forms of spermatozoa from which selection is made in response to environmental necessities." At that time nothing was known of the maternal chromosomal number, so that it was natural for McClung to reason that the monosome was a paternal chromosome not represented in the female.

In the same year Sutton (1902) described for *Brachystola* that "twenty-three is the number of chromosomes in the male cells, while twenty-two is the number I have found in the female cells, and thus we seem to find a confirmation of McClung's suggestion that the accessory chromosome is in some way concerned in the determination of sex." Subsequent studies have shown that Sutton was wrong in his count of the oogonial chromosomes.

Then Stevens (1905) found in *Tenebrio* "that in both somatic and germ cells of the two sexes there is a difference not in the number of chromatin elements, but in the size of one, which is very small in the male and of the same size as the other nineteen in the female. . . . The small chromosome itself may not be a sex determinant, but the conditions in *Tenebrio* indicate that sex may in some cases be determined by a difference in the amount or quality of the chromatin in different spermatozoa." In 1906 she wrote: "The scheme also assumes either selective fertilization, or, what amounts to the same thing, infertility of gametic unions where like sex chromosomes are present"; and in 1909*a*: "The only other alternative in these insects seems to be that sex is already determined in the egg before fertilization, either as a matter of dominance, or as a result of maturation, and that fertilization is selective . . . but any such general application is premature until adequate evidence is at hand to prove that

the sex character is represented in the chromosomes." Yet in another paper (1909*b*) that appeared simultaneously, Stevens writes: "As to the fact that the lagging chromosome of the aphids is a heterochromosome intimately connected with the phenomenon of sex determination, the present investigation of the male germ cells, I think, leaves no doubt."

Independently of Miss Stevens, and simultaneously, Wilson found that where there is a single monosome in the male, it is represented by a pair in the female; and where a large and small idiochromosome in the former, there is a pair of large ones in the latter. In his first two studies (1905*a*, *b*) he expressed himself guardedly as to such chromosomes being associated with sexual phenomena. In his third study (1906) he gives a much fuller discussion, and it is this treatment more than any other, that has aroused general interest in the subject. He mentions as one alternative that merely quantitative difference in amount of the chromatin may be the determining factor, but he criticises this for the reason that in *Nezara* the idiochromosomes are of equal size in both sexes, while a series of intergradations are known between such a condition and the one where these elements are dissimilar in size in the sexes. Therefore he maintains the alternative view, that the allosomes have qualitative differences that are sex-determining, with Mendelian dominance, and with selective fertilization. "The general interpretation . . . must include the assumption that there are two kinds of eggs (presumably in approximately equal numbers) that contain respectively the male and the female-determinant, and that the former are fertilized only by spermatozoa that lack the heterotropic chromosome (*i. e.*, the male determinant) and *vice versa*. . . . Such a selective fertilization is therefore a *sine qua non* of the assumption that the heterotropic chromosome is a specific sex-determinant." In this argument Wilson makes use of Castle's (1903) hypothesis that sex follows the rule of Mendelian segregation and dominance. To this view he adheres also in his fourth and fifth studies (1909*a*, *b*). But in his last paper (1909*c*) he opposes the Mendelian interpretation, because selective fertilization is improbable. He brings as another objection the case of the bee; here the egg after two maturation divisions

forms a male if not fertilized, a female if fertilized; under the hypothesis the female tendency should be derived from the spermatozoon—"a *reductio ad absurdum;* for the male is derived from an unfertilized egg which has by the hypothesis eliminated the female tendency."

Nowlin (1906) and Boring (1907) analyzed the phenomena of the allosomes in Hemiptera and Coleoptera respectively, and Jordan (1908) in an orthopteran, all comparing chromosomal numbers in the female and male cells, and all inclined to regard the allosomes as sex-determinants. Morrill (1909) found that in *Protenor* and other species all the cleavage cells of one individual have either thirteen or fourteen chromosomes, *i. e.*, all either a single allosome or a pair, conformable with Wilson's results on the germ cells.[1]

Payne (1909) has accepted Wilson's views of a qualitative sex-determination by allosomes; while von Baehr (1909), in discussing the subject at length, is inclined towards the quantitative explanation. Wallace (1909) concluded that in *Agelena* some spermatozoa have two allosomes, others have none, and argued that a male would result when an ovum is fertilized by a sperm with these two elements; it should be noted, however, that the different describers of aranead spermatogenesis have reached quite conflicting results.[2]

Baltzer (1909) on echinoids, drew attention to the occurrence of allosomes (idiochrosomes) in the female line, though he studied the chrosomomes only in the pronuclei and cleavage cells, not in the growth period of the oöcytes. "We distinguish accordingly two types of eggs: some with and some without an unpaired element. The chromosome number is in both cases eighteen. Therefore, we must conclude that the unpaired chromosome in the egg type where it is wanting is represented by a rod-shaped element. The sperms—always with eighteen elements—are all alike. According to the discoveries on insects, it is not improbable that the determination of sex, which would lie with the female, is connected with this difference of the egg nuclei."

[1]Some writers have denominated this case "*Wilson's Protenor-type,*" evidently ignorant that the spermatogenesis of this form was rather fully described by me (1901) some years before Wilson published his studies.

[2]Cf. the papers of Wagner, Berry, Bösenberg and myself.

Boveri (1909*a*) refers to Baltzer's work as indicative of sex-determination by particular chromosomes (the short hook-shaped ones). But in opposition to Wilson's explanation, he does not believe that one chromosome has a male and the other a female tendency, but that they differ only in activity: the larger allosome would give to a cell a larger power of assimilation, and such a view "would perhaps be qualified to serve as a basis for a general theory of sex-determination." Boring (1909) described the occurrence in the eggs of *Ascaris megalocephala* of a fifth, small chromosome element, but was unable to decide whether this is "a chromosome unit in itself, or a fragment of one of the long chromosomes," and considered it merely possible that it might be sex-determining. Boveri (1909*b*) holds this occasional structure to be undoubtedly sex-determining, a chromosome unit that is in most cases attached to the end of one of the others. At the same time he reports the occurrence of a monosome in the spermatogenesis of *Heterakis*, and concludes, in the sense of Wilson: "Fertilization of an egg by a sperm with five chromosomes leads to development of a female, by a sperm with four elements to production of a male."[1]

Finally, in the last paper on this subject, Edwards (1910) describes allosomes in the spermatogenesis of certain individuals of *Ascaris megalocephala*, and does not hesitate to call them sex-determinants.

B. Previous Criticisms of the Hypothesis.

In the preceding lines we have endeavored to state, in all brevity, the nature ofthe arguments advanced to prove that particular allosomes produce one sex or the other by their presence or absence,whether by qualitative or quantitative differences. Now we may consider certain objections that have been raised to such interpretations.

I have remained skeptical with regard to these hypotheses, though I have described many cases of allosomes in a succession

[1]On p. 135, Boveri refers to "his" discovery in 1904 that "the tetrads of the first oöcyte division of *Ascaris meg. bivalens* can consist of two shorter and two longer rodlets," whereas this was (1904) particularly described and figured by me earlier in the same year.

of papers, and have expressed myself only once on the matter, in 1906, in considering Wilson's contention which I regarded "a very plausible conclusion, but there are in particular two phenomena which must be explained before it can be accepted. One is, how an allosome becomes lost in the spermatogenesis; and the other is, how the allosomes introduced by the spermatozoön into the ovum behave during the ovogenetic cycle; on both of these questions we know as yet practically nothing."

Gross (1904) objected to the hypothesis of McClung, (1) that it is not proven that accessory chromosomes are absent in females, and (2) the case of the bee, where males develop from unfertilized eggs. He also believed that the spermatozoa with monosomes may be incapable of fertilization; but failed to note that such supposition could not be applied to sperm with idiochromosomes.

Foot and Strobell (1909) urged that the theory of the individuality of the chromosomes is not proven—today, a decided minority view. They also held that the allosomes of *Euschistus* are not chromosomes at all, and are variable in number, which is in direct opposition to the discoveries of Wilson and myself. "In the case of *Euschistus* we are told that the larger of the two chromatin nucleoli of the spermatocyte is the homologue of the accessory chromosome of other forms, and if this interpretation is correct we may expect to find a large bivalent or two univalent chromatin nucleoli in the growing oöcytes." But they find no such bodies in oöcytes, and therefore conclude that the chromatin nucleoli of the male are never transmitted to the egg, are not chromosomes at all, and hence cannot be sex-determining; this objection to the Wilson-Stevens theory is inadmissible.

An explanation suggests itself to me why allosomes, which all evidence leads us to believe must be transmitted to eggs by fertilization, act in a different way in the oögenetic cycle. That is, in spermatogenesis the single monosome, or the pair of unequal idiochromosomes, behave differently from the other chromosomes, remaining dense and compact in the growth period of the spermatocytes, probably because they are there unpaired (monosome) or of unequal size (idiochromosome), while all the other chromosomes are paired, and the two of each pair seemingly alike. In the growth period of the oocytes, on the contrary, the

allosomes of the spermatocytes seem to be represented by a pair of elements similar in all respects to each other; there is no dissimilarity of the pair, hence no conspicuous behavior different from that of the other chromosomes (autosomes). In other words, it is the singleness of the elements (monosomes), or their disparity in size and activity (idiochromosomes), that may be a reason why the allosomes behave so peculiarly in spermatogenesis. For these various considerations the arguments of Foot and Strobell against the hypothesis are not valid, though these investigators are quite right in seeing the necessity of comparing the oögenesis.

Buchner (1909) has entered other arguments against the determination of sex by allosomes, an hypothesis that he wholly rejects. He refers to their limited occurrence, which shows they could not be universal sex-determinants. Then to the occurrence of an accessory chromosome in the oögenesis, paralleling that in the spermatogenesis of *Gryllus*, he calls particular attention, insisting that it is necessary for the Wilson-Stevens theory that sperm-cells alone should have allosomes. "In *Gryllus* there can be no talk of a sex-determining function, and thereby naturally also not in the other animals with accessory chromosomes."[1]

Gutherz (1909) has, however, combated Buchner's opinion that the chromatic body in the oöcytes of *Gryllus* is a chromosome, and shows that it differs from such in many details. He also finds that "the diploid chromosome group of the male numbers 21, that of the female 22 chromosomes." "The doctrine

[1]There are a couple of points in which Buchner seems to be in error. He states (p. 409) that Goldschmidt had argued in 1904 that the allosomes might represent "trophic chromatin," and accuses me of having in 1906 overlooked Goldschmidt's priority. As a matter of fact, I wrote in 1901: "Thus it might be that in the insects the chromatic nucleoli are those chromosomes which either exert a greater metabolic activity than the other chromosomes, or which carry out some special kind of metabolism." It was Goldschmidt who had overlooked my earlier statement of this view. Then on p. 415, Buchner concludes that "the accessory chromosome (monosome) is no unit body, as has been generally assumed up to this time, but a bivalent with non-equivalent components." But in 1901, and again in 1905, I argued that the larger monosomes of sperm cells may be bivalent elements, the pair that is separate in the oögenesis represented by a pair in fusion in spermatogenesis.

of the connection between heterochromosomes and sex-determination is accordingly not disturbed by this discovery."

Morgan (1909, 1907) classes the theories of sex determination by chromosomes as qualitative and quantitative, and inclines to the latter view—he being the first to take this stand positively. By a quantitative interpretation he does "not mean that the female is simply male plus something else, a view recently advanced by Castle, but that male and female are two alternate possibilities of the living material, which possibility is realized depending on quantitative factors. . . . The gametes are not, therefore, male and female, but contain certain factors which, when combined, give rise, in an epigenetic fashion, to one or the other alternative." In the phylloxerans, the "loss of certain chromosomes from the male egg appears to follow, not to precede the size relation. . . . But there is nothing in these facts that shows that the effects are directly quantitative rather than that observable quantitative differences accompany, or follow in some cases, more profound changes." He considers as the most serious objection to the qualitative interpretation "that although the hypothesis is ostensibly based on the presence of certain chromosomes which are assumed to be male and female determining respectively, yet to these chromosomes, which are to all appearances identical, are ascribed exactly opposite functions." Morgan's whole attitude is rather hostile to the view that particular chromosomes are sex-determinants, and his arguments against the view are the most cogent yet presented.

C. Further Criticisms of the Hypothesis.

In a previous treatment (1906*a*) of the phenomenon of sexuality, I was led to define it (p. 85) as "essentially the condition of difference obtaining between conjugating individuals. . . . Because conjugation is a process distinct from reproduction, sexuality, being intimately associated with conjugation, has no primary connection with reproduction. . . . The genesis of sexuality has been this: that out of a state where all individuals were equally capable of reproduction a condition of division of labor has ensued, inducing morphological and chemical differences, between individuals capable of reproduction and conjugation and other individuals

capable of reproduction and conjugation alone. This holds true in the Metazoa, both for the germ cells and for the persons, and the male is characterized by his power to conjugate or fertilize, the female by her power to reproduce. A microgamete in the Protozoa, or a spermatozoön or male person in the Metazoa, is an individual that has lost the power of reproduction in becoming specialized for the act of conjugation. Sexuality is then the state of occurrence of dissimilar conjugating individuals, and the essential point in this dissimilarity is that only one kind of these individuals has the power to reproduce. This simple interpretation was entirely overlooked by Geddes and Thomson in their theory of 'The Evolution of Sex.' ".

The germ cells are then not without sex, as Morgan would have us believe, but have an actual *sexuality* with respect to each other, an ovum being female and a spermatozoön male; as well as a *prospective* sexuality with regard to the kind of individual they may engender. We are here concerned with the question of the determination by particular chromosomes of prospective sexuality.

Further, a hermaphrodite is bisexual, and its egg therefore prospectively bisexual, engendering both kinds of gametes. This indicates that an egg may contain potentially the characters of both sexes, or better stated, that both states of sex may arise from the same egg. This may also be true for species that are not hermaphrodite, for a female individual frequently shows certain male characteristics, and a male certain female qualities, even if in a more or less latent condition. These conditions indicate that an egg does not contain prospectively one sexual state to the exclusion of the other, but rather that maleness and femaleness are closely associated phenomena that may interchange within the same individual; a possibility suggested by Morgan (1909).

Bearing these ideas of the value of sex in mind, the following main objections may be made to the hypothesis that particular allosomes act as sex determinants:

1. While the phenomena appear to admit of a simple explanation in cases where there are only a pair of idiochromosomes, or a single monosome, in the spermatogenesis, often the conditions

of the allosomes are so much more complex than this, allowing so many different chromosomal combinations in the spermatozoa, that the interpretation of what spermatozoa are male-producing and what are female-producing becomes very difficult. Attention may be drawn, for example, to a case in the Hemiptera described by me (1901, 1906*b*). In spermatocytes of *Calocoris rapidus* there are: "twelve autosomes that divide in both mitoses, two diplosomes that do likewise (therefore are probably also bivalent), a smaller monosome that does not divide in the first but does divide in the second mitosis, and a larger monosome that divides in the first but not in the second mitosis." Other complex associations of allosomes have been described by McClung and Payne. Were the allosomes sex-determinants, we would have to conclude that in certain species a considerable number of the chromosomes subserve this end, which would be allotting an undue amount of the nuclear material to this purpose.

2. In all plants, with the exception of one (*Salomonia*) described by Cardiff (1906), and in may animals, no allosomes are known, yet these species have sexuality. It is probable that such structures will be found in certain cases where they have may been overlooked; yet they are apparently absent in some cases where special search has been made for them; accordingly, at the most they can be sex-determinants in only a limited number of cases.

3. In certain species there is the phenomenon of two sizes of eggs, some larger that produce females, others smaller and male-producing. This is known for the Phylloxerans, Rotatoria and *Dinophilus apatris;* another case has been described for an acarine by Reuter (1907); and I have shown (1907) that there are two sizes of eggs in the aranead *Theridium*, though I did not raise these eggs to determine their prospective sex values. These two kinds of eggs may be produced by the same individual, or (Rotatoria, Punnett, 1906) by different individuals. These eggs become distinguishable in the growth period, and for the Phylloxerans Morgan (1909) has shown that the egg is "sexually determined" before the formation of the polar bodies. Malsen (1906) held for *Dinophilus apatris* that the "difference between male and female eggs apparently lies chiefly in the greater or less number of fusing ovogonia"; but his brief description and few figures do

not prove this point. But however these differences arise they are clearly present early in the growth period, which is strong evidence that they cannot be produced by any sorting of allosomes in fertilization. And it is quite possible, as Beard has reasoned, that a distinction of male and female eggs may be a general phenomenon, though not usually associated with dimegaly.

4. In parthenogenesis sex is necessarily determined without fertilization; from such eggs of Rotatoria, aphids, Phylloxerans and daphnids both males and females develop. Since there is no fertilization the daughter individuals should have the same chromosome complex as the parent, should all be females, were sex determined by particular chromosomes. Else there should be anticipated separations of particular chromosomes in definite manners, which would seem to imply most complex mechanical movements; as yet we know nothing definite of such movements.

5. In hermaphroditic species an egg gives rise to a bisexual individual, never to a unisexual. Were there sex determination by particular combinations of allosomes in the fertilized egg, we would necessarily expect occasional unisexual individuals to result. In *Sagitta* Stevens (1905) found an allosome in the spermatogenesis, but neither in oögenesis nor in the first cleavage; and she, as Cardiff (1906) who described one in the plant *Salomonia*, points out that such an element can have no sexual value in these hermaphrodite species.[1]

6. As Wilson and others have realized, to regard particular allosomes as direct sex-determinants logically necessitates selective fertilization. Until a case of selective fertilization has been demonstrated, however, the discussion on this point had better be tabled.

7. Morgan has urged that it may be the mass rather than the quality of the chromosome substance that may be sex-determinative, provided that such substance is determinative at all. It is the general rule in insects that the male has less chromosome substance than the female, in having a single monosome, or a small and large idiochromosome in the place of two large ones. It might then be argued that such allosomes, by the difference in mass which they occasion, establish the prospective sex value.

[1]It is not actually proven that these bodies are of chromosomal nature.

This agrees with the fact that eggs which have given off both polar bodies and are not fertilized give rise to males, as in the Rotatoria (Whitney) and some Hymenoptera.[1] However, this does not necessarily imply that particular chromosomes are sex-determinative even quantitatively, but that the mass of all chromosomes collectively may be determinative.

8. The hypothesis neglects the part that other substances, such as the cytoplasm and the mitochondria, may have in sex determination.

9. The strongest objection to the hypothesis of particular chromosomes being specially sex-determinative remains to be discussed, and it may equally well be made against certain current explanations of heredity in general. There can be little question, at least in the present state of our understanding, that chromosomes are of great importance in cellular metabolism, and even evidence that they are in part enzyme masses. But these chromosomes, while preserving their continuity from generation to generation, which I hold to be abundantly established, are in no sense independent units, but parts of a larger whole, the "nuclear element," composed of the sum of the chromatin and linin. Further, this nuclear element is not an independent unit, but only a part, even if it be the most important part, of the cell whole. Thus the idea is erroneous to speak of the chromosomes as automatic units, for they are but parts of the cell or cell complex. The whole, as Whitman (1893) argued, cannot be the single cells or parts of them, but the entire inclusive organization. For the organism acts as a whole, not simply as the sum of many parts; it is the interrelation of the activities of the many parts, added to these, that constitutes the behavior of this major unit.

Now to assume that particular chromosomes alone are sex-determinants is to disregard this complex inter-activity. At the

[1]It is now fairly well established that drones of the honey-bee, hornet, wasp and ant all possess the reduced number of chromosomes, and therefore must have originated from unfertilized eggs that had produced two polar bodies. The work of Meves (1907, 1908), Mark and Copeland (1906, 1907), Lams (1908) and Schleip (1908) is thoroughly corroborative of this conclusion. But this does not prove that in the Hymenoptera all unfertilized eggs give rise to males, for there seem to be certain established records of females resulting from unfertilized eggs, which cases have been collected from the literature by Wheeler (1903) and Shull (1910).

most we are justified in concluding only that the chromosomes have a share in the establishment of sex. He would be rash who would venture to claim that a particular chromosome determines excretion, another determines locomotion; yet these processes are relatively simple compared with that of sexuality, which some have contended may be controlled by a particular chromosome. The hypothesis is too naïve, it assumes too great simplicity of the cell, it tastes too strong of rigid predetermination.

The idea of unit characters, promulgated mainly by the work of Mendel, DeVries and their followers, is largely to blame for such hypotheses. It seems to me that physiological study has sufficiently demonstrated that there are no actual unit characters, and it is but natural that physiologists have refused to accept them. In the analysis of cross-breeding, the investigator has to focus his attention upon one or but a few characteristics of the organism; he has to close his eyes to the great multitude of characteristics, for they are too numerous for any one mind to grasp at once. The characters he may select for examination are his units of study, and he is entirely justified in speaking of them as unit characters, provided he does not forget that they are merely arbitrary units of convenience. But most hybridists have gone further than this; they have sought to directly compare such arbitrary excerpts with units of organization, scarcely pausing to consider what is a unit of organization. Surely it is the organism as a whole that is the only unit, and just as surely all its parts are most complexly interrelated. The living body is a unity, not a colony.

Modern Mendelian explanations represent a determinant theory far more rigid and complex than that of Weismann, though, strangely enough, most Mendelists in the inception of their studies were unsympathetic to Weismannian interpretation. This is the most curious instance of how men have come to identify an arbitrary term of convenience with a part of the living organization.

When Sutton (1903) pointed out that the paternal and maternal chromosome series parallel in their pairing and separation phenomena of alternative inheritance, thus seeming to present a cellular basis for Mendelism, and Castle (1903) argued that sex

follows such inheritance, the thought originated of identifying unit characters with chromosomes. It was made to appear that unit characters are present in the germ, though just what relation a rose comb has to a particular chromosome was not elucidated. Such a concatenation of ideas as this naturally led to the identification of "sex-units" with certain chromosomes.

The better founded idea that the organism behaves as a whole, whether it be a germ cell or a multicellular body, should make us hesitate to localize any particular function solely in one particular structure, for that would mean to disregard the importance of interrelations of parts. Thus when we find particular chromosomes in one sex and not in the other, it by no means follows that these are the cause of the sex difference. All we can say at the present time is that the two phenomena are coincident. Thus I am inclined to agree with Morgan's (1909) closing thoughts: "The accessory (chromosome) may follow sex or be associated with other differences that determine sex, rather than be its sole cause."

In all probability the activities of the chromosomes are influential in establishing sex, but not in the crude way in which the process has been imagined.

One point is quite clear, that fertilization is not necessary for the establishment of sex, for any unfertilized egg that develops furnishes a sexual individual. At the same time sex may be changed by fertilization; thus Whitney (1909) has shown it to be probable that the male eggs of Rotatoria furnish males if not fertilized, but females when fecundated. Sex is then established before, but may be changed by fertilization. This clearly implies that maleness and femaleness are not unchangeable unit characters, as does also the fact that an individual of one sex may develop some of the characteristics of the other sex, a phenomenon so apparent in the human body. Maleness and femaleness would appear to be two modes of one process, the process of germ cell production, not radically different conditions. In other words, there is no valid reason to interpret sex as an immutable unit character resident in or presided over by particular chromosomes, and sorted out and distributed by Mendelian segregation with all the complex mechanisms of dominance and

determiners; but rather as a growth, the result of a labile process which may be changed by a variety of influences.

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EXPERIMENTS WITH CHRYSOMELID BEETLES.[1]

ROBERT W. HEGNER.

Results obtained by the writer within the last five years from experiments upon *Calligrapha bigsbyana* and other chrysomelid beetles have shown that the eggs, larvæ, pupæ and adults of these insects are admirably suited for the study of many of the external and internal factors of development. The eggs may be definitely oriented with regard to the future position of the embryo quite easily; they continue to develop when subjected to extremely violent mechanical conditions; and various parts may be removed without retarding their development (Hegner, 1908*a*, 1908*b*, 1909*a*, 1909*b*). The larvæ are easily reared in the laboratory in an environment similar to that which they encounter in nature; they usually thrive well under experimental conditions. The same things may be said of the pupæ and adults.

It is the writer's intention to present in this paper, and those that are to follow, the data and conclusions derived from experiments dealing with the growth and external and internal factors which influence the development of the various stages in the life history of certain chrysomelid beetles. Thus far the willow beetles, *Calligrapha bigsbyana* and *C. multipunctata*, have received the largest share of attention, but other species have also been employed.

I. The Normal Rate of Growth of *Calligrapha Bigsbyana*.

1. *The Weight of Developing Eggs.*

Method and Data.—Two series of weighings were made to determine the loss of weight of developing eggs of *C. bigsbyana*. Table I. shows the results for twelve eggs laid by four different beetles at practically the same time. Two batches of two eggs each, one of three, and one of five were taken at 1 P.M. on June 29, placed in a watch glass, and covered by another watch glass. The loss in weight is quite striking. The pigmentation of the

[1]Contributions from the Zoölogical Laboratory of the University of Michigan, No. 128.

embryos became visible through the chorion on the sixth day (July 5). However, the eggs did not hatch, as is usually the case, on the fifth or sixth day (Hegner, 1908*a*), nor on the seventh or eighth day. Loss of water no doubt prevented the larvæ from breaking through the chorion.

TABLE I.

WEIGHINGS MADE OF 12 EGGS OF *Calligrapha bigsbyana* LAID BY 4 BEETLES AT 1 P.M., ON JUNE 29, 1909. AVERAGE TEMPERATURE, 23°C.

Date.	Total Weight in mgs.	Average Weight per Egg in mgs.	Total Loss in mgs.	Average Loss per Egg in mgs.
June 29	9	.75		
June 30	8	.6666	1	.0834
July 1	7.2	.6	.8	.0666
July 2	6.7	.5583	.5	.0417
July 3	6.1	.5083	.6	.05
July 4	5.8	.4833	.3	.025
July 5	5.1	.425	.7	.0583
July 6	4.4	.3666	.7	.0583
July 7	3.8	.3166	.6	.05

TABLE II.

WEIGHINGS MADE OF 22 EGGS OF *Calligrapha bigsbyana* LAID BY 5 BEETLES AT 4 P.M. ON JULY 7, 1909. AVERAGE TEMPERATURE, 25° C.

Date.	Total Weight in mgs.	Average Weight per Egg in mgs.	Total Loss in mgs.	Average Loss per Egg in mgs.
July 7	15.2	.6909		
July 8	15	.6818	.2	.0091
July 9	14.8	.6727	.2	.0091
July 10	14.1	.6409	.7[1]	.0318
July 11[2]	13.5	.6136	.6	.0273

It has been my custom to keep a small piece of filter paper, moistened with distilled water, in the watch glass with the eggs to prevent desiccation. Too much moisture frequently enables a fungus to establish a growth upon the chorion, sometimes checking the hatching of the eggs, whereas too little moisture may also prevent hatching. In nature the eggs are laid on the under surface of leaves where they are kept sufficiently moist by the condensation of water vapor at night.

[1]The moistened filter paper was removed on July 9th and returned on July 10. This accounts partly for the comparatively great loss in weight during this interval.

[2]Twenty of the eggs hatched on July 12. The chorions from which the larvæ escaped weighed 1.176 mg., or .0588 mg. per chorion.

The eggs whose egg weights are recorded in the second series (Table II.) were placed in a covered watch glass along with a piece of filter paper which was moistened with a drop or two of water every day. The loss in weight of these twenty-two eggs during embryonic development was not nearly so great as was that of the first series (Table I.), and doubtless represents more closely the state of affairs under normal conditions. Twenty of the twenty-two eggs hatched on the fifth day, the usual time for eggs of this beetle.

Discussion and Conclusions.—The belief has been held for many years that eggs diminish in weight during the early embryonic stages, and before extraneous food is consumed. That this belief is well founded has been proved by careful experiments with the eggs of several species of animals.

Pott and Preyer (1882) have shown that the hen's egg loses weight during incubation. The amount of oxygen absorbed by the eggs equaled the amount of CO_2 excreted. This excretion, produced in the physiological processes taking place during incubation, does not, at least in this case, account for the loss in weight, as is usually supposed, since the decrease is equalized by the absorption of oxygen. The conclusion was reached that a gradual evaporation of the albumen caused the loss in weight.

When hens' eggs are incubated in desiccators the rate of development is accelerated during the first three days, but later is retarded, and many of the embryos become abnormal or die (Féré, 1894).

Eggs that develop in water have also been used to determine the loss in weight of developing eggs during development (Ritter and Bailey, 1908). Bailey used for his experiments the eggs of the California mud-fish, *Fundulus parvipinnis*. Starting ten days after ensemination, 93 eggs were weighed at intervals of about 20 hours, covering a period of 9 days. Of the 36 weighings made, only 10 showed a gain, and this was accounted for by the presence of dirt upon the eggs. Bailey believes that the "loss in weight must have been due to carbon dioxide (CO_2) and organic salts representing the albuminoid loss, which had passed out through the egg-membrane and been washed away in the sea-water."

A loss of energy also takes place during segmentation, and, in

the case of the sea-urchin egg, has actually been measured, though not enough experiments were performed to make the resultant figures of much value (Spaulding, 1907).

The eggs of chrysomelid beetles differ in several respects from any thus far used for weight experiments. In the first place they are covered by a chitinous chorion which is comparatively impervious to fluids, and is especially well adapted to withstand desiccation. The method of cleavage, *i. e.*, superficial, differs from that of the eggs heretofore examined.

The results of the two series of weighings recorded in Tables I. and II. prove conclusively that there is a loss in weight, and that this loss is largely due to evaporation. A comparison of the data in Tables I. and II. shows this quite clearly, since the eggs weighed in Table I. were allowed to develop without the addition of moisture, and consequently decreased in weight more rapidly.

2. *The Rate of Growth of Larvæ, Pupæ and Adults.*

Method and Data.—The twenty larvæ that hatched from the eggs used in determining the loss in weight of developing eggs (see Table II.) were weighed daily until they pupated; the pupæ were then weighed daily, and finally the adults. These weighings extended over the period from July 12 to August 14, 1908. Because of the daily disturbances made necessary by the weighings, many of the larvæ died. This mortality was greatest during the first four days; however, under normal conditions, many of the larvæ die during this early stage.

The data obtained have been arranged chronologically in Table III. Fig. 1 gives the curve showing the daily increase in weight and Fig. 2 gives the curve showing the daily percentage increments in weight.

Discussion and Conclusions.—The problem of growth is one of great interest to zoölogists, and its study has been given added impetus by the work of Minot (1891, 1907). This investigator considered growth not as in increase in size or volume, but as an increase in mass or weight. The rate of growth was measured by him by taking the increase in weight during a definite period and expressing it as a percentage of the weight at the beginning of that period. Any change in weight can thus be shown by successive percentages for equal periods of time.

TABLE III.

THE RATE OF GROWTH OF LARVÆ, PUPÆ AND ADULTS OF *Calligrapha bigsbyana.*

Date 1909.	Age in Days.	No. of Larvæ.	No. of Pupæ.	No. of Adults.	Total Weight in mg.	Average Weight in mg.	Daily Increase in Weight in mg.	Per Cent. Daily Increase.
July 12		20			11.3	.565		
July 13	1	20			12.	.6	.035	6.2
July 14[1]	2	20			12.6	.63	.03	5.
July 15[2]	3	17			19.	1.118	.488	77.4
July 16[3]	4	9			15.6	1.733	.615	55.
July 17	5	9			17	1.889	.156	9.
July 18	6	9			34.2	3.8	1.911	101.1
July 19[4]	7	9			39.4	4.377	.577	15.2
July 20[5]	8	8			44.6	5.575	1.198	27.3
July 21	9	8			58	7.25	1.575	28.2
July 22	10	8			107	12.375	5.125	70.7
July 23[6]	11	8			134	16.75	4.375	35.3
July 24	12	8			140.5	17.582	.832	4.9
July 25	13	8			193.5	24.187	6.605	37.5
July 26	14	8			287	35.875	11.688	48.3
July 27[7]	15	7			294.8	42.114	6.239	17.4
July 28[8]	16	7			284	40.57	— 1.544	— 3.6
July 29[9]	17	6			249	41.5	.93	2.2
July 30	18	6			235	38.917	— 2.583	— 6.2
July 31	19	6			257	42.833	3.916	10.
August 1	20	6			257	42.833	0	0
August 2	21	6			259	43.166	.333	.77
August 3	22	6			250	41.666	— 1.5	— 3.4
August 4	23	6			236	39.33	— 2.333	— 5.6
August 5	24	6			239	39.833	.5	1.2
August 6	25	4	2		242	40.33	.5	1.2
August 7	26	3	3		238	39.66	— .67	— 1.6
August 8	27	3	3		235	39.166	— .5	— 1.2
August 9	28	2	4		234	39.	— .166	— .42
August 10	29	2	4		235	39.166	.166	.42
August 11	30	2	4		234	39.	— .166	— .42
August 12	31	1	2	3	231	38.5	— .5	— 1.25
August 13[10]	32		2	3	194	38.8	.3	.77
August 14	33		2	3	190	38.	— .8	— 2.06

[1]Larvæ began to feed.

[2]Three larvæ died.

[3]Moulting began on the fourth day; eight larvæ died.

[4]Second moult in progress.

[5]One larva died.

[6]Third moult in progress.

[7]One larva died.

[8]Feeding practically stopped and larvæ prepared for pupation.

[9]One larva died.

[10]One larva died.

Minot's results from weighings made of guinea-pigs show that the growth rate increases almost immediately after birth, the decline being very rapid at first, but less rapid as the age of the

animals increases. That there is a corresponding prenatal decline in the rate of growth was shown by means of rabbit embryos. Curves representing the change in the rate of growth with age

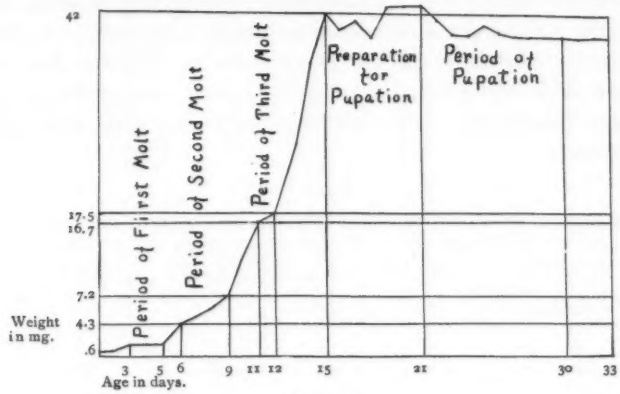

FIG. 1.

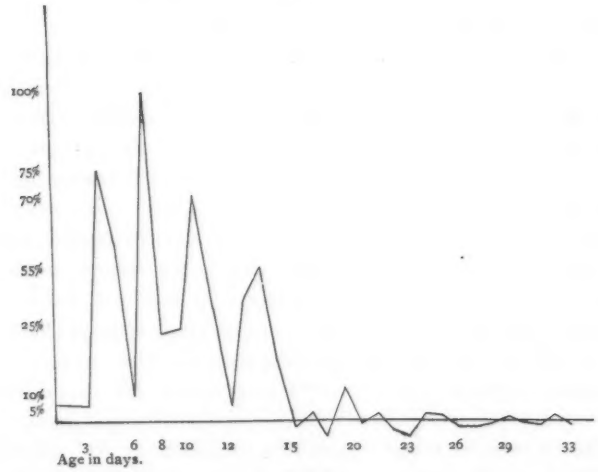

FIG. 2.

have been constructed for the embryos and young of many animals, and almost without exception the growth-rate declines as development proceeds.

Davenport (1897) has shown for the tadpoles of *Rana*, *Bufo* and *Amblystoma*, that, during the first two weeks of larval life, growth is largely due to the absorption of water, which increased from 56 to 96 per cent. During later development, however, the storing up of formed substances is mainly accountable for their growth. The curve of the growth-rate for tadpoles does not agree with the general rule; it rises first, then declines, and finally rises again. This result is probably due to the absorption of water.

TABLE IV.

THE RATE OF GROWTH OF LARVÆ OF *Telea polyphemus* (TROUVELOT, 1867).

Age in Days.	Weight in Grains.	Increase in Weight in Grains.	Per Cent. Increase.
Just hatched	.05		
10	.5	.45	900
20	3	2.5	500
30	31	28	933
40	90	59	190
56	207	117	130

Trouvelot (1867) has given a few weighings of the larvæ of the moth, *Telea polyphemus*. These have been arranged in Table IV. so as to show the actual increase in weight, and also the percentage increments for ten day intervals. The decline in the rate of growth is not regular, probably because of the meager data, but it is no doubt similar to that exhibited by the guinea-pig and other animals.

Fig. 1 shows the weight of developing beetles of the species *C. bigsbyana* from the time of hatching to the emergence of the adults, a period of 33 days. The following data will make clear certain irregularities in the curve. The larvæ usually devour a part or all of their cast-off egg-shells soon after hatching, but do not begin to feed actively until the second day; this accounts for the very slight increase in weight during the first two days. An actual decrease in weight would be expected at the moulting periods, when food-taking ceases and the chitinous covering is shed, but all larvæ do not moult at the same time (see Table V.). and instead of a decrease in the average weight, there is a slight increase. This is shown in all of the moults. The period of most rapid increase is that between the fifth and the fifteenth

TABLE V.

THE WEIGHT OF INDIVIDUAL LARVÆ OF *Calligrapha bigsbyana* WHEN 7 DAYS OLD; JUST AFTER THE FIRST MOULT.

Date of Moulting.	Weight in mgs.
July 16	5.8
July 16	5.4
July 16	5.4
July 17	4.8
July 17	4.6
July 17	4.
July 18	2.6
July 18	2.
July 19	.9

days. From the latter time onward the larvæ gradually cease feeding and lie on their backs in the earth provided for them. During this preparation for pupation, and during the period of pupation, there is a steady decline in weight until the adults emerge.

Fig. 2 shows the daily percentage increments in the weight of the developing beetles. The remarks made in explanation of Fig. 1 also explain the irregularities in this curve. The percentage increments decline very rapidly during the moulting periods. If all of the larvæ moulted at the same time, the rate would be negative.

The data obtained from these weighings confirm what Minot (1891, 1907) has found to be true of guinea-pigs, *i. e.*, the rate of growth declines rapidly during the early stages of development and more slowly during the later stages. Jenkinson (1909) has obtained similar results for many other animals by using the data already available in literature.

II. THE EFFECTS OF LIGHT UPON THE DEVELOPMENT OF *Calligrapha Bigsbyana*.

1. *The Influence of Darkness.*

Method and Data.—Experiment C.B. 42. Eight eggs of *C. bigsbyana* were laid at 12 M., June 10. Four were allowed to develop in an ordinary stender dish (7 cm. in diameter), and the other four were placed in a similar receptacle which had been covered externally with a coat of opaque paint. The same amount of moisture was supplied to each dish, and the temperature did not vary a degree.

Two eggs in the light and all of those in darkness hatched on June 19; the two remaining in the light hatched on June 20. On June 23 two of the larvæ in the darkness moulted. Three of those in the light died on June 23; the other moulted on June 24, as did the two remaining in the dark. All of the larvæ were accidentally destroyed on June 25.

Experiment C.B. 70. Four batches of eggs were laid by four different beetles at approximtaely 10:30 A.M. June 26. One half of each batch were allowed to develop in the light several feet from a window; the other half were placed in darkness as in experiment C.B. 42. The conditions of moisture and temperature were similar in the two dishes. The data have been arranged in Table VI.

TABLE VI.

DATA RECORDED IN EXPERIMENT C.B 70, SHOWING THE RATE OF DEVELOPMENT OF EGGS, LARVÆ AND PUPÆ OF *Calligrapha bigsbyana* IN WHITE LIGHT AND IN DARKNESS.

Date 1909.	White Light.	Darkness.
June 26	15 fresh eggs from 4 batches	16 fresh eggs from 4 batches
July 1—8 A.M.	5 hatched	5 hatched
July 1—1 P.M.	2 hatched	2 hatched
July 2	7 larvæ	2 hatched
July 3	7 larvæ	9 larvæ
July 4	7 larvæ	8 larvæ alive 1 larva dead
July 5	1 moulted	
July 6	4 moulted	6 moulted
July 7	1 second moult 1 still in 1st instar	1 dead
July 8	2 second moult 1 still in 1st instar	1 second moult 1 dead
July 18	5 ready to pupate	4 ready to pupate
July 21	2 pupæ	1 pupa
July 22	4 pupæ	4 pupæ
July 23	5 pupæ	5 pupæ
July 26	5 pupæ	6 pupæ
July 28	2 adults	1 adult
July 29	3 adults	3 adults
July 30	5 adults	5 adults
August 1	5 adults 2 larvæ did not pupate	5 adults 1 adult did not emerge

Discussion and Conclusions.—The eggs of *C. bigsbyana* are attached to the under surface of the leaves of the food plant of the larvæ, *Salix longifolia*, and are thus never exposed to the direct rays of the sun except for exceedingly brief intervals when the leaves twist in the wind. They develop therefore in light of

moderate intensity. Eggs that develop within an opaque mother, or that possess an opaque envelope, pass through their embryonic stages in darkness; but there can be no doubt that the chorion of the beetle's egg allows the light to penetrate, since, as I shall show in a later paper, sunlight has a decided influence upon embryonic development.

In certain cases experiments have seemed to prove that darkness delays the growth of the eggs or larvæ, *e. g.*, Yung (1878) recorded not only a retardation in the development of frog larvæ, but also a high death rate. The same investigator noted a slight retardation in the development of the eggs of the snail, *Lymnæa stagnalis*, when placed in the dark.

Vernon (1895), on the other hand, found that echinoderm larvæ suffer very little, if any, change from the normal when reared in absolute darkness. Loeb (1896) also has brought forth evidence proving that darkness does not retard the embryonic development of the fish *Fundulus*, but does effect a decrease in the number of pigment cells on the yolk-sac.

In other cases, darkness does not hinder the growth of the embryo or larva, but fails to stimulate the hatching process. Przibram (1906) found that the larvæ of the praying mantis, *Sphodromantis bioculata*, are retarded if the cocoon is placed in the dark.

In discussing experiments C.B. 42 and C.B. 70, the normal rate of development and its variations must be noted. Records of over 2,000 eggs of *C. bigsbyana* and the closely allied species *C. multipunctata* give 5 days and 16 hours as the average hatching period (Hegner, 1908*a*). This period varies according to conditions of moisture, temperature and probably other external factors, from 4 to 7 days. Records were also made of over 1,000 larvæ. The average larval life is 20 days; but, as in the case of the hatching time, this period may be shortened to 17 days or extended over 24 days by differences in external conditions. The average pupal period is 12 days, though adults frequently emerge in a shorter time, and a few do not escape until 13 or 14 days have elapsed. These variations in the duration of the different stages may occur in eggs, larvæ or pupæ from different batches of eggs or from the same batch.

The data from experiments C.B. 42 and C.B. 70 indicate that darkness has no retarding nor accelerating influence upon the embryonic development, upon the rate of larval growth, or upon the period of pupation.

One other conclusion that may be arrived at from these experiments is that darkness has no effect upon the coloration of the eggs, larvæ, pupæ or adults of the species studied. Frequent examinations were made during the growth of the beetles reared in the dark, but no variations from the normal were discovered that could be attributed to the absence of light. This confirms Przibram's (1906) results for the praying mantis, the entire post-embryonic development of which was carried out in the dark without producing any effect upon the coloring.

2. *The Influence of Colored Lights.*

Method and Data.—Experiment C.B. 64. This experiment is the only one attempted with a view to testing the effects of colored lights upon the embryonic development of beetles' eggs; but it indicates that color has no very striking influence upon the rate of development.

Several eggs from a single batch of 15, which were laid at 10:30 A.M. on June 24, were placed in each of six cylindrical tubes. These tubes were then closed with rubber corks through each of which were inserted a thermometer and a tube for ventilation. These cylindrical tubes were then immersed in different colored liquids prepared according to Yung (1878). The colors used were red, blue, yellow, green and violet, and a tube was kept in pure water as a control. The temperature in the different tubes was practically identical. The eggs in the white, yellow, green and red lights hatched on June 29; those in the violet and blue were ready to hatch on the same day, but were prevented by fungus growths.

Discussion and Conclusions.—Many experiments have been performed with eggs of a number of species of animals to determine the influence of colored lights upon their development. Yung (1878) used freshly laid eggs of the frog, *Rana esculenta* and *R. temporaria*. At the end of two months all of the tadpoles in the green light were dead, those in the white and yellow lights

were greater in number, those in the red light were retarded and finally died, and those in the violet light were larger, but less advanced and had greater powers of resistance.

These results have not been confirmed for the frog and other animals by later investigators. For example, Vernon (1895) found that the larvæ of echinoderms, in some cases, were not killed by the green light, and that yellow light caused greater injury than red. Driesch (1892), moreover, claims that the eggs of *Rana*, *Echinus* and *Planorbis* are not influenced by any of these colors.

In experiments on the praying mantis, Przibram (1906) found that the influence of green, red and yellow glasses was unfavorable, though this may have been due to differences in the temperature, which was not controlled.

My experiment with the eggs of *C. bigsbyana* confirms for the eggs of this beetle the results obtained by Driesch for eggs of *Rana*, *Echinus* and *Planorbis*.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF MICHIGAN,
April 15, 1910.

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THE MARSUPIUM OF THE UNIONIDÆ.

GEORGE LEFEVRE AND WINTERTON C. CURTIS.

In a recent preliminary announcement of a new system of classification of the Unionidæ which is based upon a study of the anatomy of the fresh-water mussels of Pennsylvania, Ortmann[1] suggests a division of the family (exclusive of the Hyriinæ) into four subfamilies, namely, Margaritaninæ, Unioninæ, Anodontinæ and Lampsilinæ, respectively. His arrangement lays especial emphasis on the characters of the marsupium and involves several important modifications of Simpson's classification, which he maintains must be recast to a considerable extent in order to make it represent the natural affinities of the group. Although it will not be possible to form an opinion in regard to the validity of the proposed changes before the appearance of his detailed results, he has undoubtedly fallen into a serious error wi h respect to the marsupium of one of his subfamilies, and it is the object of the present note to point out this mistake.

In connection with the fresh-water mussel investigations which have been under way in this laboratory for some time and which have been carried on for the United States Bureau of Fisheries, primarily for the purpose of determining the feasibility of artificial propagation of the Unionidæ, we have had occasion to give attention to the anatomical and histological structure of the marsupium in a large number of genera, and, furthermore, we have been particularly concerned with the changes that occur in the gills during the period of gravidity. Since a fundamental discrepancy exists between Ortmann's description of the gravid gill in his subfamily Anodontinæ and our own observations on at least three of the genera which he includes in this group, namely, Alasmidonta, Anodonta and Symphynota, we have thought it advisable to call attention to the fact.

Ortmann (p. 117) makes the following rather astonishing statement concerning the structure of the marsupium of the Anodon-

[1] "A New System of the Unionidæ," A. E. Ortmann, *Nautilus*, XXIII, February, 1910, pp. 114–120.

tinæ: "Water-tubes in the gravid female *divided longitudinally into three tubes*, one lying toward each face of the gill, the third in the middle; only the latter contains eggs or embryos, and is much larger than the other tubes. This division into three parts is not present in the sterile (*sic*) female." Although it is not specifically stated, it is to be inferred from the above description that the divisions of the water-tubes into three parts is due to

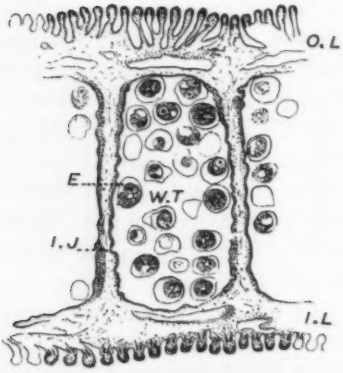


FIG. 1. *Anodonta cataracta* Say.—Horizontal section of portion of gravid marsupium, showing a water-tube, undivided and filled with embryos. *O.L*, outer lamella of gill; *I.L*, inner lamella; *I.J*, interlamellar junction; *W.T*, water tube; *E*, embryos, x31.5 Kline del.

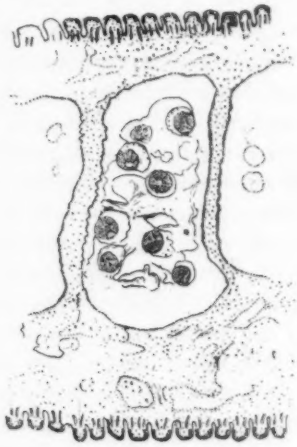


FIG. 2. *Alasmidonta truncata* Wright.—Horizontal section of portion of gravid marsupium, showing a water-tube, undivided and filled with embryos. The mass of embryos is somewhat contracted into the middle of the tube. x31.5 Kline del.

the presence of longitudinal partitions running parallel with the lamellæ, but no intimation is given as to how they arise, or how they disappear after the marsupium has discharged its contents. To any one familiar with the structure of the gills of the Unionidæ the statement that the water-tubes exhibit a temporary division into three parts is on its face improbable, for it would be difficult to imagine how such a division could be brought about, and still more difficult to understand why, when once established, it

should disappear after spawning. It is true that one occasionally encounters a partial fusion of two adjacent interlamellar junctions, with a consequent division into two or more parts of the water-tube lying between them, but this is not constant in occurrence for the species and, when it is present at all, it involves only a single tube here and there in the gill. We have observed such fusions in a few individuals belonging to different genera in both gravid and non-gravid gills, but it is a condition that must be regarded merely as an occasional variation and is entirely different from that which is supposed by Ortmann to exist in the Anodontinæ. His description, moreover, is at total variance with our observations in the three genera referred to, as sections of the gills in these forms, taken at various stages during the gravid period, show *absolutely no trace of such a division of the tubes*. In Figs. 1–3, which are drawn from horizontal sections of the gravid marsupium of *Anodonta cataracta* Say, *Alasmidonta truncata* Wright and *Symphynota complanata* Barnes, respectively, the water-tubes, containing embryos and glochidia, are seen in their usual form, undivided longitudinally and bounded by the inner and outer lamellæ of the gill and by he interlamellar junctions. If such a division of the tubes into three parts, as Ortmann describes, were present, it would of course be indicated in these sections.

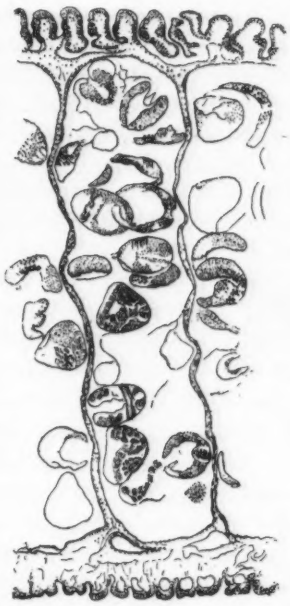


FIG. 3. *Symphynota complanata* Barnes.—Horizontal section of portion of gravid marsupium, showing a water-tube, undivided and filled with glochidia. x 31.5 Kline del.

We are at a loss to understand what appearances observed by Ortmann could have given rise to his error. The only thing that suggests itself is that the material which he used had been

badly preserved and the gills in consequence much shrunken. In this event, it is quite possible that the embryos might have been contracted into a mass in the middle of the water-tube and the mucus, by which they are surrounded, coagulated in such a way as to cause the appearance of septa stretching between the interlamellar junctions when observed under a low magnification. It is not uncommon to find the embryos contracted in this manner to a greater or less degree as a result of fixation, as may be seen in Fig. 2, in which the mass of embryos has been withdrawn slightly from the inner surface of the lamellæ. The fact that he states that the divisions are only present in the gravid gills would lend some degree of plausibility to this explanation.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF MISSOURI,
April 26, 1910.

THE "PYLORIC GLAND" OF THE ASCIDIAN BOTRYLLUS—AN ORGAN OF EXCRETION?

HAROLD SELLERS COLTON,

UNIVERSITY OF PENNSYLVANIA.

INTRODUCTION.

This contribution deals with the anatomy and physiology of an organ found in most of the groups of the Tunicata. This organ is usually composed of a system of fine tubes which ramifying over the walls of the intestine, are joined to some portion of the stomach by one or more ducts. Although of general occurrence in the Ascidiacea and Thaliacea, yet there has been no concurrence of opinion as to its function. In consequence of this fact hardly two authors have referred to it by the same name. Hence we have this organ figured as (1) glandes diverses (Savigny, 1810)[1] and referred to, if we leave out literal translations as, (2) liver (Hancock, 1866) (Krohn, 1852) (Milne-Edwards, 1841),[2] (3) glande annexe du tube digestif (Chandelon, 1875) (v. Winiwarter, 1896), (4) glandola epato-pancreatico (Della Valle, 1881), (5) lacteal system (Lister, 1834) (Huxley, 1851) (Ritter, 1896), (6) intestinal gland (Herdman, 1882) (Maurice, 1888), (7) lacunes stomaco-intestinales (Roule, 1884), (8) glande stomacale (van Beneden et Julin, 1884), (9) darmumspinnende Drüse (Seeliger, 1882) (Dahlgrün, 1901) (Isert, 1903), (10) organe réfringent (Giard, 1872) (Pizon, 1893) and (11) glande pylorique (Lacaze-Duthiers et Delage, 1889) (Willey, 1893).

With such a choice of names what shall we call the organ in question? Since the name pyloric gland is short and sufficiently non-committal and has been dignified by usage, we will use it in preference to the others.

The following study is an outgrowth of one that the writer has been at work on for the past two years, and as it has developed into slightly other lines than was originally planned, he has taken this excuse to make a separate paper of this portion. The work

[1]See Chandelon, 1872.

[2]See Giard, 1872*a*.

as a whole was begun in the zoölogical laboratory of the University of Pennsylvania. It was continued at the Zoölogical Station at Naples, at the Fisheries Laboratories at Woods Hole, and Beaufort, and the following part of it completed in the Zoölogical Laboratory of the University of Pennsylvania. At this point the writer wishes to express his great thanks to the Carnegie Institution for the use of one of their tables at the Naples Laboratory, to the authorities of the station for their many kindnesses and hospitality, to the United States Commissioner of Fisheries for the use of a table for two weeks at Woods Hole, and one for two weeks at Beaufort, and also to the directors of those stations, Dr. F. B. Sumner and Mr. H. D. Aller in particular.

Although for this study most of the living material was procured in the salt-water tanks of the vivarium of the University where *Botryllus* colonies have been established for many years, yet the wealth of material preserved in Naples and Woods Hole has often been called into requisition while living material of other families of ascidians were studied at Beaufort.

According to Bancroft ('03) there is but a single species of *Botryllus* found in the north Atlantic Ocean and its extensions. Many have been described, but they are found to be based on color variations and habit of growth depending partly on the age and partly on the physiological state of the colony. The writer having worked at both Naples and at Woods Hole supports the view of Bancroft and considers that *Botryllus schlosseri* (Pallas) Savigny, is the form represented on both sides of the ocean.

The material was fixed in Flemming's solution, in corrosive sublimate, sublimate acetic, formol, etc. The best results were procured with Flemming's solution. Sections were cut 6 μ and stained in Delafield's hæmatoxylene and eosin. However, most of this study was made on the living animals and sections were used only to check up the results.

Morphology.

The alimentary tract of *Botryllus* is of the typical ascidian type and may be represented by the letter U of which one arm will be the œsophagus and stomach while the other is represented

by the intestine and rectum (Fig. 1). In the angle between the two arms lies a small blind sac, an out-pocket from the pyloric end of the stomach. Its walls are thick and glandular, similar to the walls of the stomach, but the cells that compose it do not contain the secretion that gives the stomach its characteristic yellow color.

This organ was called by Lahille ('90) the pyloric cœcum. It is into this sac, at the point where it enters the stomach, that the duct of the pyloric gland empties.

If we follow the duct from the point where it enters the pyloric cœcum and trace it to the intestine we will find it divide just before reaching that organ, sending a branch both to the right side and to the left side. At once on reaching the walls of the intestine both branch many times, finally ending in blind bulbs or ampullæ. However, all the branches do not end thus, but a few—not more than five or six—proceed half way to the anus, often without branching again. These tubes do not end in ampullæ. As they reach the region of the rectum in some cases, we will for convenience refer to them as rectal tubules.

It is very easy to study the organ in the living *Botryllus* and indeed it is possible in that way to see much more than can be observed in the preserved material, either in sections or in surface view. When a cormus is removed from its substratum and p'aced on a microscopic slide, the stomach and intestine can be teased out with a pair of needles under a dissecting microscope. The cormus is then removed from the slide, a drop of sea water added and the whole covered with a cover glass. The writer found artificial light in the shape of a Welsbach burner, a Zeiss apochromatic 2 mm. objective, and compensating oculars 8 × and 12 ×, necessities in the present study.

In the living tissue the tubes and bulbs appear highly refractive. In some cases it is quite easy to see the nuclei and even the chromatin in the nuclei. In many cases cell boundaries are quite clear and the presence of cell granules is easy to determine.

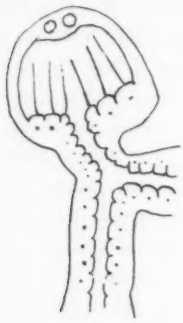
The ampullæ are bounded by a rather flat epithelium, the cell walls of which contain often refractive granules of a yellow color. The cells bear long whip-like flagella, but it is difficult to determine if all are so provided. These flagella soon lose their move-

ment after being placed on a slide, although the writer has observed them beating for five hours after the alimentary tract was removed from the organism. Fig. 2 represents the character of the epithelium and flagella in two adjoining ampullæ.

In *Botryllus* this organ was reported ciliated by Della Valle ('81) who wrote, p. 458: "La struttura intima di questa glandola è simplicissima trattadosi d'un semplice epitelio, che io ho veduto sempre sfornito di cigli vibratili." He gives no figure.

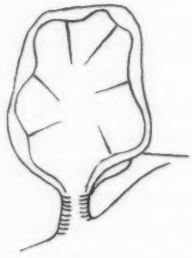
Pizon ('93) particularly mentions that he is unable to verify Della Valle and finds the lumen of the ampullæ unciliated. There are three other cases in which the pyloric gland has been found ciliated—Chandelon ('75) in *Perophora*, text Fig. 1, Uljanin ('84) in *Doliolum*. text Fig. 2 and Isert ('03) in *Microcosmus*. The

FIG. 1.



After Chandelon.

FIG. 2.



After Uljanin.

Ampullæ of the pyloric gland as seen in *Doliolum* and *Perophora* respectively.

latter found the ducts and believed the ampullæ were ciliated too, although he could not see it. The cases in which cilia have been seen in the ampullæ were all observed before the modern methods of microscopic technique were evolved. Since then we have been carried away by the use of dead material when in many cases, perhaps, as much if not more could be seen in the living.

Although the protoplasm of the walls of the ducts and ampullæ seems to be clear and refractive in the living animal, yet there are here and there yellowish granules in the cells. These are exceedingly minute, .1 to .2 μ in diameter, and are found in that portion of

the cell nearest to the lumen of the gland. One rarely sees more than a single granule in a cell. Among these small granules are larger ones, .5 to .8 μ, distinguished from them by being more refractive. In character they seem much like the brown concretions found in certain blood cells (Fig. 18). However, these concretions cannot be found in the preserved material, so the supposition is that they are composed of a material that is not coagulated by the killing fluid, this material being of the nature of a secretion. The concretions in the blood cells are not easily dissolved by any ordinary reagents used in microscopic technique therefore they are found in the preserved material.

At Beaufort the writer had the good fortune to be able to examine the ampullæ of the living *Amaroecium stellatum*, *Perophora veridis*, *Ascidia* sp., *Styela plicata* and *Molgula manhattensis*. All had flagella in the pyloric gland. In *Amaroecium* the flagella were similar to those found in *Botryllus*. In *Perophora* some ampullæ were like those found in *Botryllus* (Fig. 22), but others had exceedingly few cilia which were directed toward the mouth of the tube and not away as in Chandelon's figure (Fig. 21). Since the writer was able to observe movement in all the cilia in this ampulla their direction can be determined to a certainty. The walls of the ampullæ of *Ascidia* showed no essential character different from that of *Botryllus* (Fig. 19). In the cells of the gland of *Styela* (Fig. 24) is found the yellow secretion just as Isert described it in *Microcosmus* and in the lumen of the gland are found globules of it. In *Molgula* the organ is typical (Fig. 23). Since so many diverse families of ascidians show flagella in the lumen of the pyloric gland, the writer believes that if carefully looked for this organ in all tunicates will be found to bear flagella.

The walls of the tubules are similar to those of the ampullæ except that the cells are more cuboid (Fig. 3), and the flagella shorter. No granules are to be detected in the lumen of the tubes of *Botryllus* such as Isert found in the ducts of the pyloric gland of *Microcosmus*, and as the writer has found in the ducts of the gland in *Styela*.

When we compare the rectal tubules with the other portions of the organ in *Botryllus* several things may be noticed. Among

these things our attention is particularly called to the relative fewness of flagella and nuclei in the terminal portions of the tubules. Figs. 4–13 show flagella while Figs. 14 and 15 show nuclei. Although it is not possible to distinguish cell boundaries either in the preserved material or in the living tissue, yet the fewness of the nuclei might suggest the possibility that the cells composing this part of the gland have intracellular lumens. Again the terminal ends of these tubes exhibit two different types. We may have those unsegmented with a very small lumen, 2–6 μ (Figs. 8–12, 14 and 15), or we may have segmented tubes with a much larger lumen, 4–10 μ. In the first type of tube our attention is at once attracted to the terminal end of the tube. In most cases there seems to be a very thin place in the walls of the tubes. This thin place may be formed in three ways: (1) by the lumen of the tube approaching the exterior (Figs. 8, 14, 15), (2) by a cup-like depression in the end of the tube (Figs. 9-12, 15), (3) by a vacuole in the wall of the tube which does not communicate either with the exterior or the interior. To these three cases there is a fourth effect that the writer has observed. He thought that he could see tubes less than a micron in diameter that formed a direct communication between the interior of the tube and the blood space. The structures found at the end of the rectal tubules are so small that what we may interpret as a duct may be nothing more than a division between two cells—or a nucleus—both of which look clearer in preserved material and in the living tissue than the cytoplasm of the cell. The cup-like depression at the end of the tube suggests the organ of Boveri as found in *Amphioxus* or perhaps a nephridial funnel. The writer has searched the neighborhood about the ends of the tubes to see if he could find solenocytes as described by Goodrich ('09) in *Amphioxus* but without result. Again he has watched particles in the blood in the neighborhood of the possible opening, yet in no case has he been able to observe such a particle enter the tube. In this connection the experiment of Kupffer ('72) is interesting. He says (p. 381): "Mir ist es auch bei *Ascidia canina* gelungen, dieses System wenigstens partiell vom Herzen aus zu injiciren. Die Injectionsmasse war in mehrere der blinden Anhänge eingedrungen. Solche blinde kolbige Anhänge sind auch nichts Neues

im Gefässsystem der Ascidien. Man findet dasselbe an den colonialen Gefässen in der gemeinsamen Tunica der Synascidien. Ich halte daher das Ganze für einen besonders enwickelten Theil des Circulationsapparates dem wohl neben der Resorption des Chymus noch andere Functionen zukommen."

If *Ascidia canina* should have open communication between the lumen of these tubes and the blood cavity it would easily explain how Kupffer found the injection mass in the lumen of the gland. Since no communication has been demonstrated, it would be easier to explain the result of Kupffer in the light of the writer's own experiments with indigo carmin on *Styela* (p. 43). With the present evidence before us we cannot assume that there is any communication between the blood spaces of *Botryllus* and the lumen of the pyloric gland.

The cases in which cilia have been seen in the ampullæ were all observed before the modern methods of microscopic technique were evolved. Since then we have been carried away by the use of dead material when in many cases, perhaps, as much, if not more, could be seen in the living.

Pizon ('93) has studied the origin of this organ both in the tadpole and bud of *Botryllus*. Of this study the writer has verified the results and can but accept the conclusion of Pizon ('93) that in both cases the origin of the pyloric gland is from the endoderm by a simple diverticulum of the gut. This agrees perfectly with Van Beneden and Julin ('84 and '86) in *Phallusia*, Lefevre ('98) in *Perophora*, Ritter ('96) in *Goodsiria*, Seeliger ('82) in *Clavelina*, Uljanin ('84) in *Doliolum*, etc. In all cases it arises as an out-pocket of the stomach.

Experiments.

The smallness of the pyloric gland in *Botryllus* and the fineness of the tubes and ampullæ as found in the larger ascidians, together with the close application of the gland to the walls of the intestine, to the reproductive organs or to the renal vesicles, would forbid, in any form that has yet been available to the writer, direct physiological determinations. It is due to this that the nature of the organ has been problematical. To be sure Henri ('03) claims to have isolated the gland in *Salpa*, but as yet the

writer has been unable to procure the form in question. As no direct experiments on the nature of the fluid contained in the ducts and ampullæ have in this case been possible, the writer has resorted to indirect means—that of the use of certain invitam stains.

In the use of these stains the writer has not proceeded far and hopes at another time to undertake a fuller discussion of their significance. Suffice it to say that certain dyes, when introduced into the blood of a living organism in solution, have affinities for formed substances in the cells of certain tissues, as methylene blue in nervous tissue. Others, such as neutral red, act as indicators, telling us whether a given substance has an acid reaction or not, while still other dyes are segregated out of the blood as solids and deposited in cavities often connected with the exterior.

Following the experiments of Chrzonszezewsky ('64), Heidenhain ('74)[1] by injecting certain dyes, principally indigo carmin and ammonium carminate, into the veins of vertebrates came to the conclusion that the former dye was excreted by the Malpighian tubules of the kidney, while the glomeruli excreted the carminate. Kowalewsky ('89) carried this idea into his experiments on invertebrates, concluding that renal cells show either acid or alkaline reaction which determines the character of the secretion. However, Schmidt ('91)[2] has shown this idea false, as both ammonium carminate and indigo carmin may be excreted by the same organ. Nevertheless, it is a rather general characteristic of renal organs that they excrete carmin in some form.

In this study of *Botryllus* the writer has placed colonies in neutral red, in Bismarck brown, in ammonium carminate and in indigo carmin, studying the reaction of the pyloric gland to these dyes. Neutral red in concentrations rendering the sea water a pale yellow, stains the secretion in the cells of the organ an intense red and colors the liquid in the lumen of the ducts and ampullæ also. The probable significance of this is that the secretion has an acid reaction. Bismarck brown coloring the water much like that of the neutral red, stains the granules brown and

[1]Cited from Bruntz, '03.
[2]Cited from Bruntz, '03.

in contrast to the blood and to the sea water, the contents of the lumes of the tubes and bulbs is very brown. There are two alternatives by which to interpret these two experiments—either these stains act as indicators or they are actually concentrated in the lumen of the organ being separated from the blood by the cells of the walls of the tubes and ampullæ. Beyond certain leucocytes located in the neighborhood of the organ which seem to collect the dye, the only other cells that take up the ammonium carminate and indigo carmin are the vacuoles in the intestinal cells. A cross-section of the intestine has the shape of a rectangle (Fig. 17), the ends of which are made up of an epithelium of flat cells bearing cilia, while the sides are thick and are composed of two sorts of columnar cells, ciliated and glandular (Fig. 14). The gland cells under ordinary condition contain a clear liquid. It is the vacuoles of these gland cells which stain slightly in ammonium carminate and indigo carmin. The significance of this will be discussed later on.

Both *Molgula* and *Ascidia* were treated with indigo carmin at Beaufort but the pyloric gland showed no reaction to them.

In *Styela*, however, indigo carmin in concentrations as shown in the table gave in every case a characteristic reaction. The material in question were rather small *Styela* 20–30 mm. The large ones required too large receptacles.

Experiment 1.		100 c.c. saturated sol. of indigo carmin in sea water.		100 c.c. sea water	4 days.
"	2	100 c.c.	do.	100 c.c. do	2 "
"	3	100 c.c.	do	100 c.c. do	4 "
"	4	200 c.c.	do		2 "

The animals in experiments 1, 2 and 3 lived, in experiment 4 they died.

When the animals were examined large blue concretions were found in the ampullæ and ducts of the pyloric gland (Figs. 25 and 26). These concretions gave that portion of the intestine covered by the pyloric gland a blue color. The writer considers that the indigo carmin was excreted from the blood into the canals of the gland.

Discussion.

Turning now to the character of the pyloric gland in other tunicates, we may as well begin with the Larvacæ. Although this organ is absent in most of the genera, it seems to be represented at least in a rudimentary form in two described by Chun ('81) from deep water of the Mediterranean Sea—*Stegasoma* and *Megalocercus*. Here we have a diverticulum of the gut which may be a possible homologue of the pyloric gland or at least to the pyloric cœcum. However, in no case is the organ developed as it is in the other orders of tunicates.

Our present knowledge of the pyloric gland in the Thaliacea and Ascidiacea can best be presented in tabular form. The table in question does not pretend to be complete but gives in condensed form the observations of various investigators.

Family.	Genus.	Authority.	No. of Ducts.	Type.	
Doliolidæ	*Doliolum*	Uljanin ('84)	1	Dendritic	Ciliated.
Salpidæ	*Salpa*	Chandelon ('75)	2	Reticular	——
Pyrosomidæ	*Pyrosoma*	Huxley ('59)	1	"Ramifying"	——
		Seeliger ('89)	1	Dendritic	——
Polyclinidæ	*Fragaroides*	Maurice ('88)	1	Reticular	Unciliated.
	Amaroecium	Author	—	——	Ciliated.
Distomidæ	*Distaplia*	Della Valle ('82)	1	Reticular	——
Botryllidæ	*Botryllus*	Della Valle ('82)	1	Dendritic	Ciliated.
		Pizon ('93)	1	Dendritic	Unciliated.
		Author	1	Dendritic	Ciliated.
Polystyelidæ	*Goodsiria*	Ritter ('96)	1	Branching	——
Clavelinidæ	*Clavelina*	Seeliger ('82)	1	Dendritic	——
		Author	1	Dendritic	Ciliated.
Perophoridæ	*Perophora*	Chandelon ('75)	1	Dendritic	Ciliated.
		Author	1	Dendritic	Ciliated.
Phallusidæ	*Phallusia scabra*	v. Winiwarter ('96)	2	Reticular	——
	Ascidia sp.	Author	—	——	Ciliated.
	Corella	v. Winiwarter ('96)	5–11	Reticular	——
Cynthiadæ	*Microcosmus*	Isert ('03)	1	Reticular	Ciliated.
	Polycarpa	Lacaze-Duthiers ('89)	1	Dendritic	Unciliated.
	Styela rustica	Wagner ('85)	1	Reticular	——
	Styela plicata	Author	1	——	Ciliated
	Styelina	Lacaze-Duthiers ('89)	1	Dendritic	——
Mulgulidæ	*Molgula* sp.	Author	—	——	Ciliated.

As far as this organ has been particularly described it is much the same in all families. The usual number of the ducts is one, but there may be more. The tubules are either dendritic or

form a network over the intestine. All the tubes, ducts and ampullæ that have been examined carefully have been found to be lined with a ciliated epithelium. Moreover, it is worthy of note, perhaps, that Huxley, ('51) and Della Valle ('81) have observed a bladder-like swelling of the duct in *Didemnum*. Todaro figures the same for *Salpa*. A type of gland differing slightly from the ones referred to above was described by Julin ('04) in *Archiascidia*. The single duct was short, and branched into but six tubules which did not branch on the intestine but ran parallel to one another almost all the way to the anus. There were no typical ampullæ. These tubes can best be compared to the rectal tubules described above for *Botryllus*.

As the writer mentioned in the introductory paragraph great difference of opinion exists as to the function of this organ. To be sure, few authors have ventured to strongly support one idea and most have been quite reserved in their conclusions, yet it is of sufficient interest to warrant the writer's reviewing their opinions briefly. Not considering those views that were based clearly on misconceptions cf. Vogt ('54), as to the structure of the organ, we will take up a few of the others. Really when Hancock ('66) called the organ in question a true liver much can be said to support the view. Its relation to the blood supply plainly recalls that of the vertebrate liver. Indeed with what we know at present of the organ, it would be very difficult to refute this idea, particularly as the vertebrate liver has been known to excrete carmin.

Chandelon ('75), Della Valla ('85) and von Winiwarter ('96) and Isert ('03) consider, after thoroughly reviewing the subject, that the function is digestive. Henri ('03) has other than morphological evidence. He says, p. 765: "En faisant des macérations de cette glande pylorique, on obtient un liquide riche en amylase, il ne digére ni l'albumine, ni la fibrine; cette macération agit au contraire faiblement sur la gélatine. Cette glande contient donc bien des ferments digestifs. Les macérations des autres parties du corps de la *Salpe* donnent des résultats négatifs."

Kupffer ('52) and Roule ('84) by means of injections arrived at the conclusion that these tubes were part of the blood vascular system. Lister ('34), Huxley ('51) and after them Pizon ('93)

and Lefevre ('98) have agreed that it probably serves an absorbing function, something like the gastrovascular canals of Cœlenterata. Huxley ('51) asked: "Does this tubular system represent a hepatic organ or is it not more probably a sort of rudimentary lacteal system—a means of straining off the nutritive juices from the stomach into the blood by which these tubes are bathed?" It is very probable that the organ has a digestive function; there seems strong evidence to support that idea. But the direction of the cilia in the duct would forbid the conclusion of Huxley, etc., that the function is that of absorption.

There is yet another function that has been attributed to the pyloric gland. Kowalewsky ('74) was inclined from what he knew of the structure of the organ in *Peropora* to attribute to it urinary functions.

Krunkenberg ('80) says: "Ich finde sie als constantes, durch die Murexideprobe leicht und schön nachzuweisendes Product der als Nieren angesprochnen drüsigen Darmanhänge bei *Phallusia mentula*." This statement is based on a misconception. He did not distinguish that the pyloric gland and the renal vesicles were not part of one system. What he analyzed were the concretions which others have found to contain uric acid. This interpretation is supported by the fact that he could not find uric acid in the gland of *Ciona* and of *Cynthia*, neither of which have renal vesicles covering the intestine.

In *Salpa*, Todaro ('01–'02) described three pairs of diverticula from the alimentary canal that had the power of taking up carmin. The first pair was in the pharynx, the second pair in the œsophagus and the third pair the pyloric gland.

Let us now turn and inquire as to what organs have been previously described as possessing the power of elimination of waste products of metabolism from the body of tunicates. Roule ('84) makes the distinction between a kidney of excretion and one of accumulation. Through the investigations of Van Beneden ('46), Kupffer ('72), Lacaze-Duthiers ('74), Kowalesvky ('89) and Dahlgrün ('00), we have a knowledge of this latter type of organ at least in a few groups. The kidney accumulation may be said to consist of two types. In *Salpa*, *Ciona* and *Botryllus* it consists of blood cells containing brownish concretions. The second type

is composed of closed vesicles lined by an nonciliated epithelium which encloses a fluid in which are suspended one or more relatively large concretions. There may be many small vesicles as in *Ascidia* and *Ascidiella*. In *Cynthia* we have a few larger vesicles and in *Molgula* a single large one.

In a kidney of accumulation, the waste matter of the organism is stored up in the form of a solid which is freed from the organism only by death. Harmer ('92) has described such an organ in the ectoprocta and the kidney of accumulation can be found in several groups of animals. In the Tunicata other organs have been described as kidneys of excretion. Julin ('91) suggested that the neural gland had perhaps an excretory function. Metcalf ('00), on reviewing the subject of the neural gland, considers that there is no evidence to support the view of Julin. Roule ('84–'85) on the other hand, described about the opening of the deferent canal in *Ciona* a mass of pigment cells which according to this author is a kidney of excretion.

To the view that the pyloric gland is a kidney, there is one serious objection. This is the fact that Henri ('03) found in the pyloric gland of *Salpa* a diastatic ferment in great abundance. This would seem to be a strong argument in favor of a digestive function for the organ were it not that such a ferment is found in the kidneys of certain mammals, sparingly in the dog it is true but richly in the rabbit (Oppenheimer, '09).

We have in certain forms of tunicates a kidney of accumulation, but in nearly all groups a pyloric gland. The Appendiculariæ which are without it are so exceedingly minute that all their tissues, if not actually bathed by the sea water, are in close proximity to it, so that the need of special organs of excretion is not quite so urgent. Be that as it may the question naturally arises, has this tunicate organ any characters in common with the excretory organs of other groups of animals?

The shape and character of the terminal bulbs, and the ducts are parallelled in part by the multicellular ciliary flames found in the Nemertinea. A section of the terminal end of a duct in *Lineus* by Punnett ('11) shows a condition very similar to that of the ascidian. Each cell of the organ bears a single flagellum which is directed away from the blind end of the tube. That the

duct of the pyloric gland opens into the stomach and is derived in development from the entoderm is a condition characterizing no other excretory system out side of the Arthopods. However, since the vertebrate liver has been shown by Chrzonszezewsky ('66) to excrete carmin, and has morphologically the same position as has the pyloric gland of the ascidian and has a similar development, there is good reason to believe that the two are homologous. Willey has homologized the organ with the hepatic cœcum of *Amphioxus* which Hammar ('93) about the same time compared to the liver of the Craniota. Can we not conceive that in the hypothetical ancestor of the vertebrate the liver arose as an organ of excretion and in the tunicate it has retained more of those characters?

Summary.

1. There are in *Botryllus* two sorts of terminations to the tubes that compose the pyloric gland, bladder-like ampullæ and long straight blind tubes—the latter we have called rectal tubules because in many cases they extend to the region of the rectum.

2. The ducts and ampullæ of *Botryllus* as well as *Ascidia*, *Styela*, *Molgula*, *Perophora*, *Clavelina* and *Amaroecium* are lined by cells bearing long whip-like flagella, the ends of which are directed toward the mouth of the duct.

3. Many of the rectal tubules have a termination difficult to interpret. This has the appearance, in most cases, of a cup-like depression in the end of the tube which seems to form a communication between the blood cavity and the lumen of the tube. In no case, however, could such a communication be demonstrated.

4. The direction in which the free ends of the flagella point indicates that the contents of the lumen pass toward the stomach and therefore the function of the organ is secretory rather than that of absorption.

5. Part of this secretion is probably found in the minute yellow globules found in the cells of the ducts and ampullæ. If these yellow globules represent a secretion, this is soluble in water and does not form masses in the lumen of the tube as in *Microcosmus* and *Styela*.

6. Bismarck brown and neutral red are concentrated in the

lumen of the organ in the form of a liquid while the indigo carmin is found concentrated in solid form in the gland of *Styela*.

7. In the tunicates in general no special kidney of excretion has been recognized. Although the gland in question may have other functions also, yet its structure and properties seem to indicate that it is the kidney of excretion of the tunicates, and is in turn homologous to the vertebrate liver.

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Description of Plates.

The figures were all drawn with a Zeiss microscope and apocromatic lenses. In making all the drawings a camera lucida and artificial light was used, in the case of *Botryllus* a Welsbach burner, and in the case of the other forms an ordinary oil lamp. With the exception of Fig. 1, Fig. 16 and Fig. 17 all were drawn at a magnification of 1,500 diameters, and have been reduced in reproduction to 1,000.

A = ampullæ.
bc = blood cell.
C = concretions of indigo carmin.
I = intestine.
Nu = nucleus.
O = Œsophagus.
PC = pyloric cœcum.
PS = peribranchial sac.
R rectum.
RT = rectal tubules.
RO = pyloric gland.
S = stomach.
V = vacuole in intestinal cell.

Explanation of Plate I.

Fig. 1. The stomach and intestine of *Botryllus*. × 52.

Fig. 2. Two ampullæ of the pyloric gland in *Botryllus*. This drawing shows the long whip-like flagella and also the granules in the cells. × 1,000.

Fig. 3. Portion of one of the main ducts. × 1,000.

Figs. 4–7. Optical section of a rectal tubule showing segmentation, granules, and flagella. × 1,000.

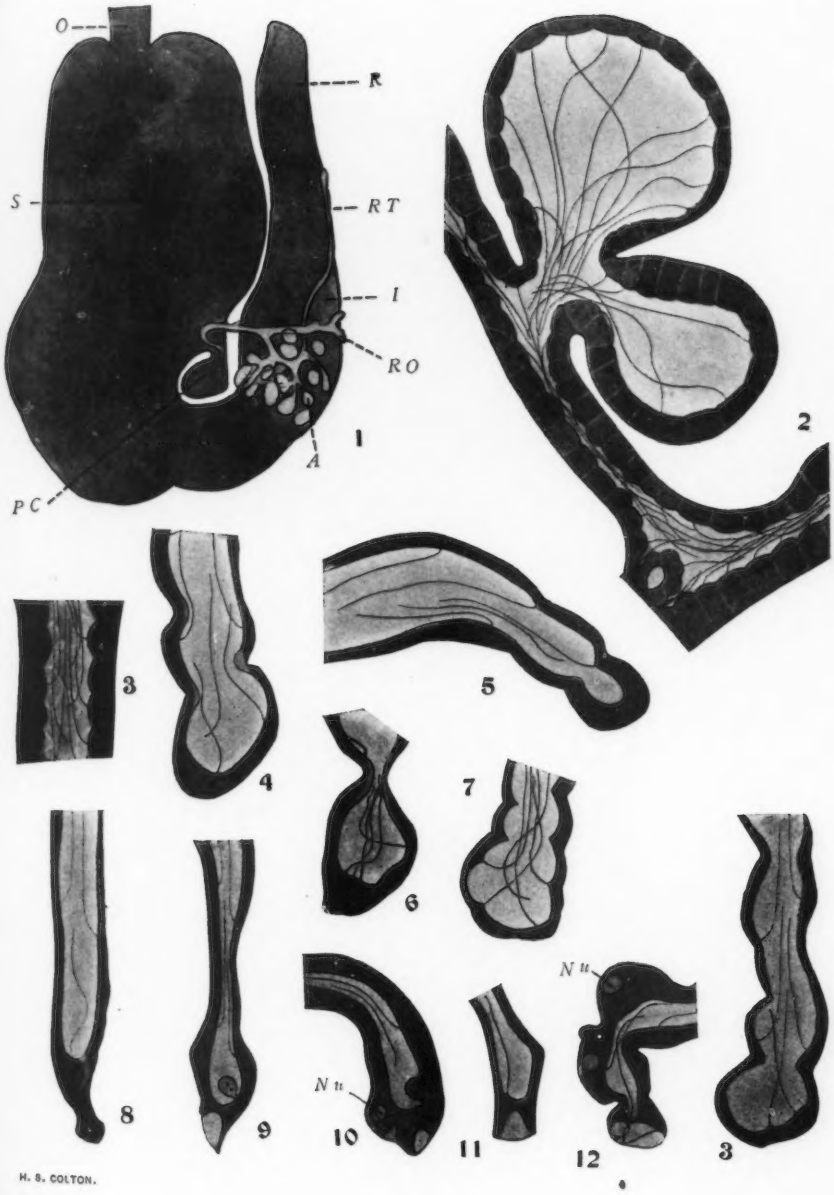
Fig. 8. Optical section of a termination of a rectal tubule showing a thin place in the wall of the tube. × 1,000.

Fig. 9. Optical section of a cup-like depression in the end of a tubule. × 1,000.

Fig. 10. Optical section of a tube with two cup-like depressions at the end. × 1,000

Figs. 11–12. Termination with a single cup-like depression. × 1,000.

Fig. 13. Optical section of a segmented tube in the walls of which there are no granules. × 1,000.



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Explanation of Plate II.

Fig. 14. Section 6 μ thick of the termination of a rectal tubule of *Botryllus* which was fixed in Flemming's solution. This shows a thin place in the wall of the tube and also a vacuole. In this figure is also shown the relation of the tube to the walls of the intestine. The vacuoles in the intestinal cells are those which stained red and blue in ammonium carminate and in indigo carmin respectively. × 1,000.

Fig. 15. Drawing made from two adjoining sections of a rectal tubule, which show terminations with both a thin place and a depression. × 1,000.

Fig. 16. A section of a bud of *Botryllus* showing the origin of the pyloric gland as an out pocket to the stomach. × 320.

Fig. 17. Sections of the rectum of *Botryllus* fixed in absolute alcohol, glacial acetic acid and chloroform. This shows the relation of the tubes to the canal. × 150.

Fig. 18. Two living blood cells, in one of which the nucleus is visible in the other it is not. × 1,000.

Fig. 19. Optical section of ampulla of *Ascidia*. × 1,000.

Fig. 20. Optical section of rectal tubule of *Ascidia*. × 1,000.

Fig. 21. Optical section of ampulla of *Perophora*. × 1,000.

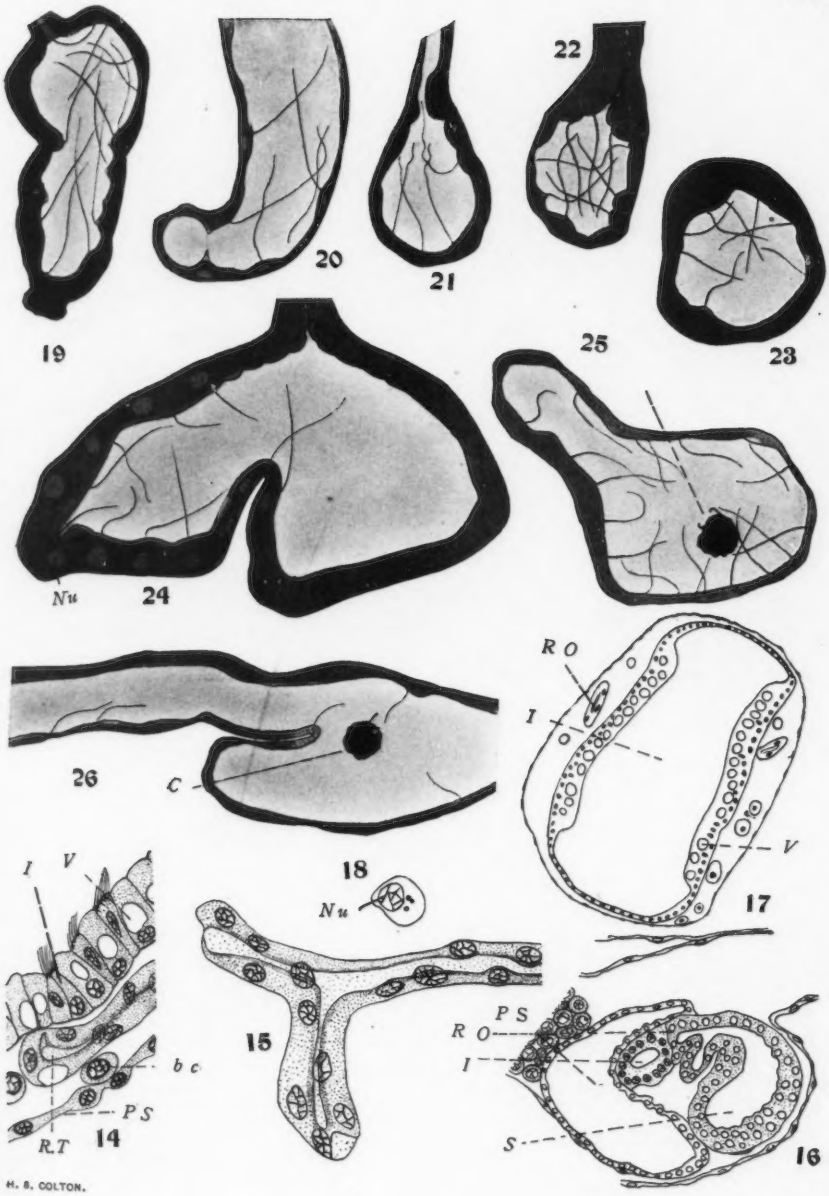
Fig. 22. Optical section of ampulla of *Perophora*. × 1,000.

Fig. 23. Optical section of ampulla of *Molgula*. × 1,000.

Fig. 24. Optical section of ampulla of *Styela*. × 1,000.

Fig. 25. Optical section of ampulla of *Styela*, containing a concretion of indigo carmin. × 1,000.

Fig. 26. Optical section of a tube of the refringent organ of *Styela* containing a concretion of indigo carmin. × 1,000.



H. S. COLTON.

THE SPERMATOGENESIS OF THE CADDIS-FLY (PLATYPHYLAX DESIGNATUS WALKER).

B. F. LUTMAN.

The numerous works that have appeared in recent years on the spermatogenesis of insects have covered practically every order and in some orders, such as the Hemiptera, many of the important families. There still remain, however, a number of the smaller orders which have not been investigated, and there is always the possibility that these may contain new or especially favorable structures. The Trichoptera is one of these small orders. The Neuroptera and Lepidoptera to which it is believed to be most nearly related have been investigated by Miss McGill (8) and by Toyama (15) and Henking (2). The small size of the larvæ and the difficulty of dissecting out the very young tests has probably deterred investigators from attacking this group when larger, better known and more easily obtainable forms were to be had in the Hemiptera and Orthoptera.

I shall not go into an extensive discussion here of the general literature of spermatogenesis. *Platyphylax* has in the main the same development that has been described for all insect forms. There are certain points in which it is different and the literature on them will be discussed in connection with my own observations.

The only paper in which the spermatogenesis of the Trichoptera is discussed at all is in that of Lubben (3). Lubben was more interested in the external morphology and the development of the testes than he was in the cytological details, but he observed a number of facts well worth noticing. The spermagonial cells arise out of the original genital cells by division. They grow into large cells which appear, united with two cyst epithelial cells. The origin of these epithelial cells of the cysts was not clear to him. Inside these epithelial cells now arises a mass of cells by division. Then by two more divisions the spermatids are produced; these have sharply bounded nuclei with clear plasma around them. The spermatids increase in length and the whole

complex of spermatozoa takes on the form of a long-drawn out cylinder. The nuclei are all arranged at one end, pointing one way, while the body of the sperm fills the remaining space. These he calls spermatocysts.

The formation of the free spermatozoids does not seem to occur either in the free or the enclosed larvæ. At the time of pupation there are only spermatocysts. In the pupa there results along with growth and the further differentiation of the spermatozoids the resorption of the walls of the spermatocysts. In the mature pupa the spermatozoa are free and lie in thick knots in each division of the testes.

The caddis-flies, as has long been known, pass practically their entire life in the larval and pupal stage. Vorheis (17), who has followed the life-history of *Platyphylax designatus*, has found that the eggs are laid in April and that the larvæ appear about two weeks later. During summer and fall the larvæ grow, and from November to January more and more larvæ are found. The period of pupation begins about the middle of February and is indicated by larger irregular stones being attached to the anterior end of the sand cases "while some are attached to the lower surface of the large rocks by a mass of silk at the anterior end." For a few days after the closing of the case the larvæ remain inactive but unchanged, before becoming pupæ.

The caddis-fly larvæ offer the advantage that while the life-history just sketched is gone through with at about the time indicated, larvæ of almost any size may be still obtained up until January and February. The material was obtained during three years; the first year from about the first of December to the first of February; the second year during the month of June, and the third year in May. The spermagonial and reduction division occur in the larval stage, so the half-grown larvæ of a length of 6–10 mm. was the material in which it was found. The sperms are apparently all formed at or soon after pupation.

In the older specimens the testes were dissected out, but in order to get the very young spermagonia it was necessary to section the entire abdomen.

Practically all the material was fixed in Fleming's weaker solution although sublimate-acetic was used on some abdomens

with very good results. The sections were stained in Heidenhain's iron alum hæmatoxylin or in Flemming's triple stain.

The Testes.

The testes, as Vorheis (17) has already noted, occur in the fourth and fifth segment of the abdomen. They are small five lobed structures usually surrounded by a mass of fat.

These five lobes or follicles are about equal size but of various shapes. In general they are somewhat wedge-shaped, the thinner part of the wedge being, of course, toward the point of attachment of the organ (Fig. 13). In longitudinal section about fifty cysts appear in each follicle (Fig. 14), the larger ones at its smaller end.

Each follicle has its own wall and the flattened nuclei of these wall cells frequently appear in the sections. In addition each cyst is provided with a wall. The youngest cells are farthest from the point of attachment of the testes. In one section, as shown in Fig. 14, cysts containing spiremes for the first division, tetrads, first and second divisions, transformations of the spermatids, and fully developed sperms occur in the order named across the section. The young cysts show in section, 6–8 spermatocytes; the older ones, where the cells are smaller, due to the two divisions and to the growth of the cysts, show 12–18. There is no definite zonation of spiremes, first divisions, second divisions, etc., such as Wilcox (19) has figured in *Caloptemus femur-rubrum*, but the general tendency is for the cysts containing the younger cells to be further away from the smaller end of the follicles. The fact that there is no definite zonation of successively older spermatocytes makes it at first confusing in picking out the sequence of development. The difference in size and number of them in the different cysts however makes this possible after a little observation. The spermatids in developing in the cysts, as is usual, have their heads all pointing in one direction as they lie side by side.

The Spermagonial Cells.

As the early history of the spermagonial cells does not seem to have been worked out on many species of insects a rather full description of that process will be given here. In this insect the

primary and secondary spermagonial cells are sharply distinguished in their division stages while the development of the cysts with their surrounding epithelial cells is almost diagrammatic in its clearness.

There seems to be a general agreement in the literature that each of the cysts that lie in the follicles of the testes has its origin from a single germ-cell. This conception seems to have originated in the work of Vallette St. George (12) on the testes of *Rana temporaria*. He found that if he teased apart the parenchyma of the testes, groups of cells, that he called spermatocysts, would drop out. These structures had walls of their own in which were imbedded one or two nuclei. St. George believed that each of these cysts arose from a single cell, one of his "Ursamenzellen." These would correspond to the last of what is now known as the primary spermagonial cells. By the division of these cells there was produced the spermacysts containing the spermagonia; or as now called, the secondary spermagonia.

Montgomery (10) describes the cysts in *Pentatoma* but did not work out their origin. The connective tissue network of the young testes contains, he believes, in each mesh a single spermagonium or at least only a few spermagonia. These cells divide and the cells produced by their division are surrounded by a cyst-membrane derived from an extension of the connective tissue investment of the follicle. The germ-cells and the cells of the cyst walls have then a different origin.

Several others, among them Henking, Paulmier and McGregor, are mentioned by Sutton (14) as having noted the arrangement into cysts and the cyst walls but none of them seems to have followed their development.

Sutton (14) in studying the spermagonial divisions in *Brachystola magna* also worked out fairly completely the development of the cysts. A cyst membrane with nuclei in it is formed even in the two-celled stage of the secondary spermagonia. The cysts assume a roughly pyramidal shape, the cells inside it largely dividing tangentially to the surface of the cyst. All the cells in one cyst seem to divide simultaneously producing by means of about eight divisions, 256 cells. The cysts are not attached to the walls of the follicles.

In all of the species so far described, however, the cysts lie so closely appressed against each other and against the follicular wall that it is impossible to decide certainly as to the identity of the cyst walls. In all of them too the number of cells seems to be large. In these respects *Platyphylax* offers a much better opportunity for deciding the question of cyst wall and cell number in a cyst.

The earliest stage of the reproductive organ that I was able to be sure was the testes was that shown in Fig. 1. At this stage there has already appeared the lobing into five divisions that is so characteristic of the mature organ. The cells composing it are apparently all of about the same size; there being at this time no visible differentiation into germ-cells and cyst epithelial cells, if such does occur. The nuclei of these cells are a little larger than those in other parts of the body. The distribution of chromatin in these nuclei is characteristic of that in all parts of the body, the pieces being of rather large size and of a rather regular number instead of having the form of fine granules. Sometimes these pieces of chromatin are in the form of bodies which might well be taken for split chromosomes, while others have the diamond shape characteristic of the tetrads with which they might readily be confounded (Fig. 3). Divisions are rather rare at this time and growth is apparently rather slow. The division figures observed were of typical mitotic type. The peculiar part that the nucleolus plays in this division will be discussed under the reduction divisions as in them it is much larger and more readily followed. All that is necessary to say here is that it seems to change its spherical shape, becomes elongated and apparently forms a chromosome.

In the testes next advanced in the stage of development the primary spermagonial cells occupy only about a third of the space while the remainder of it is filled with the secondary spermagonial cells in various stages of division to form the mature cysts can be followed at every stage.

The testes have grown considerably. The five divisions are fully formed; they have acquired distinct cellular boundaries; and the original secondary spermagonial cells, instead of being rather closely packed in the testes, are now lying with consider-

able free space between them. This free space is what makes the task of following them, and their boundaries, so easy. About two thirds of the primary cells, spermagonia, or "Ursamenzellen" as they are variously called, have divided to form groups of 2, 4, 8, 16 or 32 cells while the other third is undivided.

A closer examination of these undivided cells will now show that in addition to the larger germ-cell there is lying closely appressed against it a smaller kidney-shaped nucleus (Fig. 4). The chromatin in both nuclei is distributed in the form of little flecks rather regular in size and number and united by strands.

The origin of this nucleus that lies in the epithelium of the cyst is not clear. There is no such differentiation in the cells in the testes at the preceding stage. If these epithelial cells were in the testes at that time it was impossible to distinguish them. It may be that it is an epithelial cell that has made its way in some fashion into the interior of the testes and there surrounded the germ-cell. It is a difficult question to decide and one on which my material gives little evidence.

The further development of the cysts of the follicle can be readily followed in the two- (Fig. 5), four- (Fig. 6), eight- (Fig. 7), sixteen- and thirty-two-celled (Fig. 8) stages. The cell-walls are difficult to distinguish at these stages as the plasma membrane which is all that surrounds the cells is very thin and the cells are pressed tightly against each other. The same difficulty was noted by St. George (12) who found, however, that he could distinguish the walls in material fixed in "quick acting reagents." At any of these stages the nucleus of the cyst epithelium will show. In the older cysts there appear quite frequently to be two such nuclei, but in the younger stages at least I have observed only one. These nuclei do not seem to divide.

The divisions in the secondary spermagonial cells are very regular, all the cells in one follicle dividing at one time (Fig. 9). All these divisions are typically mitotic. Sutton (14) found that the division spindles were largely tangential to the walls of the cyst, owing to pressure in those directions. This caused the cyst to grow in length. As the cysts of the caddis-fly are spherical, the divisions occur in equal numbers in all planes. It can be readily determined that there are regularily 32 cells in each cyst of the

follicles, which indicates, of course, that five divisions have occurred.

After the last division, the cells of these mature cysts grow and the nucleus becomes much larger in proportion to the size of the cell. Fig. 8 shows the size of the cyst immediately after it has acquired its full number of cells and Fig. 10 shows a cyst after the growth period in which the cells are in the spireme for the reduction divisions. There are therefore two growth periods: one after the last primary spermagonial division and the second before the reduction divisions.

The Reduction Divisions.

After the last spermagonial divisions the growth is apparently very rapid. The nucleus is very large, occupying almost the entire cell and the chromatin is in the form of regular little pieces (Fig. 11). The remains of the spindle of the last spermagonial division lies near the nucleus in the form of an ovoid body, the nebenkern.

Apparently the first stage in division shows the little pieces of chromatin drawn out until they resemble chromosomes while the nucleolus becomes oval or spindle-shaped (Fig. 12). The pieces of chromatin become united by a slender connection and seem to spin out and lose their identity. The nucleolus in the meantime becomes more and more elongated (Fig. 15).

The chromatin now seems to go into a long slender spireme, the mass of the threads lying at one side of the nucleus and occupying about one half of it. This is apparently the stage of synapsis. The strands are very fine and delicate and as the nuclei are not so very large, it is impossible to make out any pairing of the threads, if such occurs. The nucleolus lies as a long drawn out body either in this tangle of the threads or on its surface. Occasional loops stick up out of the denser clump but they are too small to follow or to make much out of. After these stages, which are apparently short as they are not numerous, the chromatin again fills the entire cell in the form of the spireme. This spireme gradually becomes shorter and thicker. This stage is a very long one as is shown by the fact that in sections where any divisions at all occur, about half of the cells are in this stage.

It may be that the stage of synapsis is much shortened in the caddis-fly and that of the spireme is correspondingly lengthened.

The spireme now breaks up into chromosomes and these lie in the nucleus as long slender paired bodies (Fig. 22). The chromosomes now come to have the peculiar shapes such as X's, Y's, twisted figures, etc., characteristic of the stage of the reduction division (Fig. 23). These soon shorten into the tetrads (Fig. 24). The tetrads take on the typical lozenge form and sometimes show an opening in their center. The manner in which these bodies arrange themselves on the spindle could not be definitely determined as they are small in size and the four segments of the lozenge are all of about the same length.

The metaphase shows a sharp pointed spindle with its extremities in a centrosome just inside the plasma membrane (Fig. 25). The telophase of this division shows the chromosomes pulled about two-thirds of the way back to the centrosome and the nuclei still connected by the remains of the central spindle (Fig. 26). The centrosome at this time is still apparently divided and the rays from it extend down to the cluster of chromosomes.

The second division, following almost immediately, has little to distinguish it in the metaphase from the first except in its size and in the size of its chromosomes (Fig. 29). In the telophase of this division the centrosome was not to be found—just what had become of it has not been ascertained. The remains of the central spindle is the most conspicuous feature of this stage. The chromosomes seem to spread out at once, as soon as a nuclear membrane is formed, and make the ordinary reticulated network of a resting nucleus (Fig. 34).

There is a period of growth after the reconstruction of the nucleus, such as Paulmier (11) and other authors have described, followed by a stage in which the nucleus shrinks. Even before the chromosomes are entirely distributed and while they are still present as little pieces of chromatin (Fig. 34), the cell begins to lengthen and the axial filament to form. In fact the long drawn out form of the cell which it has from the last division does not seem to change but passes over at once to the young sperm. The transition stage takes a long time for its completion and any number of transition stages can be found. The chromatin col-

lects more or less to the outside of the nucleus, forming a hollow sphere, just inside the nuclear wall. The remains of the spindle lie near the nucleus as an oval body in a clear zone. This body apparently divides as Baumgartner (1) has described and a part of it seems to pass around the nucleus. Soon after these stages shown in Fig. 34 the nuclei become very sensitive to the fixing reagents and as a consequence practically all of them have collapsed. This would probably be the stages when the chromatin is in the form of a tube with a very small lumen. The fully developed sperm is shown in Fig. 36.

The details given above are in the main the same as have been described many times for animal spermatogenesis. In the action of some parts of the cell mechanism there is always quite a little variation and it is to these parts that particular attention will be paid. The two structures to be especially noted are the centrosome and the "chromatin nucleolus."

The Centrosome.

It is impossible to locate the centrosome except when it is occupying a position at the end of the spindle. At any other place it is impossible to say whether a certain dark staining body is a centrosome or not. Bodies appear near the nucleus where the centrosome should be and have all the characteristics of centrosomes, being darkly staining little granules surrounded by a clear space and frequently with what appear to be rays running up to them, though these latter are very faint and small, but the multiplicity of such bodies makes it impossible to trace the history of the centrosome with any certainty for any distance or even to be sure that such a centre is present at all times. At the ends of the spindle, its position is of course always easy to determine. It usually first appears so as to be definitely recognized at the tetrad stage as a dark body on the plasma membrane. It appears as a very small black granule on which the fibres terminate and lies just inside the plasma membrane and apparently attached to it. It is most conspicuous during the metaphase when the sharp pointed spindle ends in this little granule at the plasma membrane. It may be, of course, that here it is not a body at all but only the common point of attachment of the fibres of the

central spindle and of the aster. Whatever the origin and nature of the centrosomes may be at this time it is at least something that will take a stain and that has a definite location. After the second division, at which time it lies at the ends of the spindle again, it seems to disappear until a dark staining granule appears at one side of the nucleus from which the axial filament seems to be growing out. I have not traced the centrosome around to discover whether the two are identical or not but from the results on other animals it undoubtedly is. From this stage on then, it would form the middle piece of the sperm.

The Chromatin Nucleolus.

McClung (4) has described in the germ-cells of certain grasshoppers a body which he calls the accessory chromosome. Previous to this discovery of this body the "chromatin nucleolus" had been described by Montgomery (10) in the Hemiptera. More recently the discussion of heterotypic chromosomes has been given special importance by the papers of Stevens (13) and Wilson (20, 21) especially in connection with the theory of sex-determinants.

The divisions in the nuclei of *Platyphylax* show a body which, while it seems to have something in common with these described structures, is in other respects quite different. Its behavior has been reserved for this separate discussion.

The various changes undergone by this body have been followed to some extent both in the spermagonial and reduction divisions. As the cells, and consequently this chromatin nucleolus, are larger in the reductions divisions it will be described there first. The nucleus of the young spermatocytes contains a nucleolus that stains typically both in the triple and the iron haematoxylin. This body is either spherical or ovoid in shape. In the preparatory stages of division it begins to lengthen and become spindle shaped. It frequently lies twisted over on itself or is spoon-shaped at this time (Fig. 15).

In a later stage when the chromatin has gone into the synaptic condition this body seems as a rule to be somewhat smaller in diameter as though it were spun out as the other chromatin has been (Fig. 16). It does not lie among the chromatin strands, but

as a rule rests outside the chromatin mass which at this time occupies about half the nucleus. At this time the body seems to be a part of the spireme. In the next stage when the chromatin comes out of synapsis this body appears as a part of the much thickened spireme thread (Figs. 17–21). It is quite large at this time and much resembles a nucleolus in its intense staining reactions but it is spindle shaped and from each end runs out the continuation of the spireme thread. In the triple stain at this time it still takes the safranin color. A still closer examination shows that the threads leading up to this body are double and the body itself is divided into two halves by a longitudinal furrow. It lies at this time, when seen in side view, as a flat spindle-shaped figure immediately pressed against the nuclear wall. It now resembles very much the accessory chromosome as drawn by McClung (6) in his Fig. 2, except that he at this period discovered no break and the body that he drew was proportionally considerably larger. He describes and figures a stage (Fig. 5) where this body goes into a spireme of its own but no split was observed in this separate thread. Considering the subsequent behavior of this body—the formation of a tetrad and of chromosomes, such a split is to be expected. A split was to be observed in this chromatin nucleolus of *Platyphylax*; its spireme is a part of the spireme formed from the remainder of the chromatin. The split in it becomes more marked (Fig. 19) and the body finally opens out as a lozenge-shaped tetrad (Fig. 21). At this time the other chromosomes have not yet formed, although the longitudinal split has taken place. In some cases it looks as though the transverse splits have already occurred, but the thread still remains intact with this body as a part of it. This black staining tetrad is one of the most conspicuous parts of the nucleus at this time (Fig. 21).

The other chromosomes are now formed and assume the peculiar shapes characteristic of them at the time before they form the tetrads. This body is still recognizable at this time on account of its regular lozenge shape while the others are in the form of X's, Y's and various other twisted shapes (Fig. 23). At the next stage, however, when all the chromosomes have become tetrads this body is indistinguishable from them (Fig. 24).

There is no evidence that it has disappeared as the nucleolus usually does; it seems simply to have become a tetrad. The elaborate formation and dividing of the tetrad would argue against this disappearance also. This chromatin nucleolus can be traced no further. During the equatorial plate stage of division the chromosomes all lie in one plane and it is impossible to identify any particular one as the transformed nucleolus. Neither does any one lag behind in divisions in the metaphase nor in the movement toward the poles in anaphase (Figs. 26 and 32). If this body forms a chromosome, as it undoubtedly does, that chromosome behaves exactly like all the others.

The number of chromosomes is of great interest here if this is a true accessory chromosome. According to either the McClung or the Wilson type of an accessory chromosome, or the Wilson type of a heterotypic one, there should appear an odd number of chromosomes plus this additional one; or as McClung has found in *Orchesticus* sixteen chromosomes and the accessory one.

In all the counts made in *Platyphylax*, however, the number of chromosomes for both the reduction divisions was found to be thirty. This is the result of repeated trials. These countings are as easy to make as of dots on a piece of paper (Figs. 27, 28, 30) as the polar plate views are numerous and the chromosomes are short. There is some variation in size in the chromosomes in polar view but it is impossible to pick out one of them as the special structure that has been followed.[1]

It will be seen from this description that while this body resembles the accessory chromosome of McClung in many respects still it differs from it in one very essential one. It apparently forms a tetrad that divides in both divisions and so each sperm would receive one fourth of it. This would make it impossible for it to serve as a sex-determinant, for all the sperms would receive a part of it, and not half of them, as would happen if we credit the observations of McClung or of Miss Wallace (18). This body in that case could not be a sex-determinant.

[1]In cutting abdomens to get the development of the testes I have cut and stained as many, or more, females than I have males. The divisions in the former can be readily observed here as they are much larger than in the testes. The nucleolus undergoes a similar lengthening out, and then forms a part of the spireme. Marshall (9) in his paper on the development of the ovary also shows several figures that strongly suggests this.

Voinov (16) has described in the divisions preceding sperm formation in a beetle (*Cybister roeselii*) a body which very much resembles in its appearance the one under discussion. He, however, did not observe it forming a chromosome tetrad, nor did he follow it during the nuclear divisions except to figure a small darkly staining body lying outside the nucleus in the cytoplasm, which he believes to be the same. It may be that this body might form a tetrad as in *Platyphylax*.

Heidenhain in "Plasma und Zelle" approves Flemming's opinion that the nucleolus is always surrounded by a thin layer of true chromatin. Some appearance such as those shown in Fig. 2 would seem to suggest at least that there might be a ring of some other material around the nucleolus but the structure is so small that it is impossible to say certainly. If this were true, however, the changes described would only mean that the nucleolus loses its form during the divisions and becomes pulled out and split, between the chromatin strands surrounding it.[1]

At any rate whatever this structure in *Platyphylax* may be, whether "accessory chromosome" or "chromatin nucleolus," some disposition of it must be made in discussing these aberrant chromosomes in the nuclei of insects.

This work was done under the direction of Prof. W. S. Marshall, of the University of Wisconsin, to whom my thanks are due not only for assistance with methods and literature but also for quite a portion of the material from which the sections were made.

Summary.

1. The development of the follicular cysts can be readily followed in this insect. Each cyst contains 32 cells derived by 5 divisions from a primary spermagonial cell and enclosed in a membrane containing one or two nuclei.

2. The reduced chromosome number is always 30; the somatic number is probably 60 from a count in the oogonial divisions.

3. The centrosome is only to be followed from the tetrad stage to the anaphase but probably forms the middle piece of the sperms.

[1] A count of the somatic number of chromosomes in the oögonial division gave 55–60. The exceedingly large number makes counting difficult and not very accurate in these divisions.

4. The nucleolus of the spermatocyte seems to form a tetrad which becomes one of the thirty of the reduced number.

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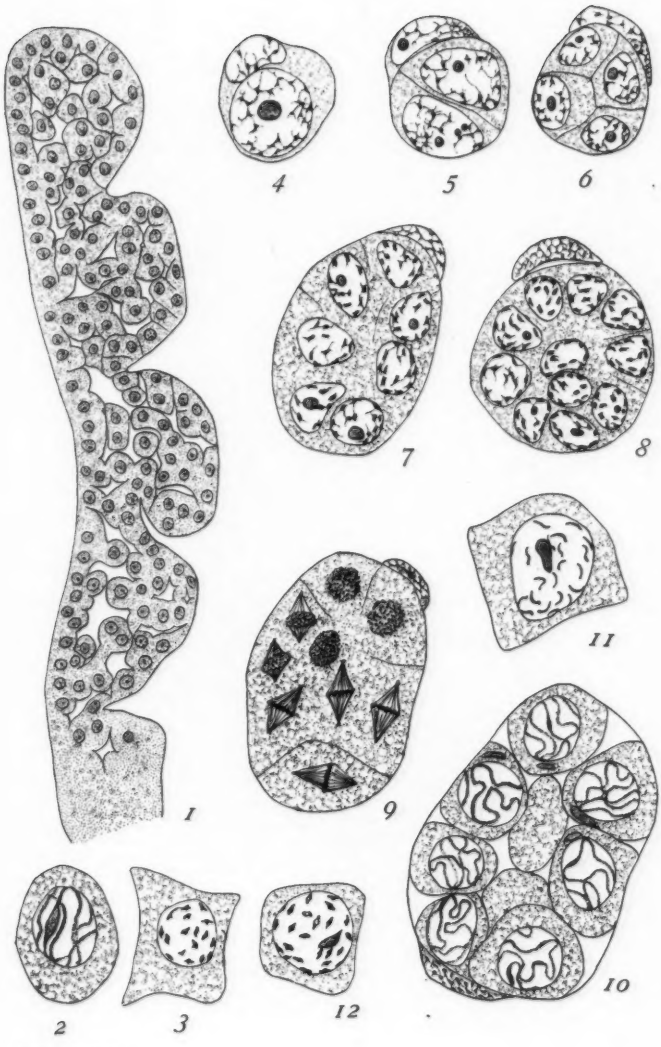
Explanation of Plate I.

Magnification. (1) 80; (13) 25; (14) 80; all the others are magnified about 1,200 diameters except No. 20 which is about 1,800.

1. Longitudinal section of very young testes showing primary spermagonia cells.
2. Spermagonial cell in spireme.
3. Resting spermagonial cell.
4. One-celled stage of cyst showing epithelial nucleus lying alongside of it.
5. Two-celled stage of cyst.
6. Four-celled stage.
7. Sixteen-celled stage.
8. Thirty-two-celled stage.
9. Sixteen-celled stage dividing.
10. Mature cyst with the nuclei all in the spireme stage; after the growth period.
11. Young spermatocyte just leaving the resting condition.
12. Young spermatocyte showing lengthening of the nucleole.



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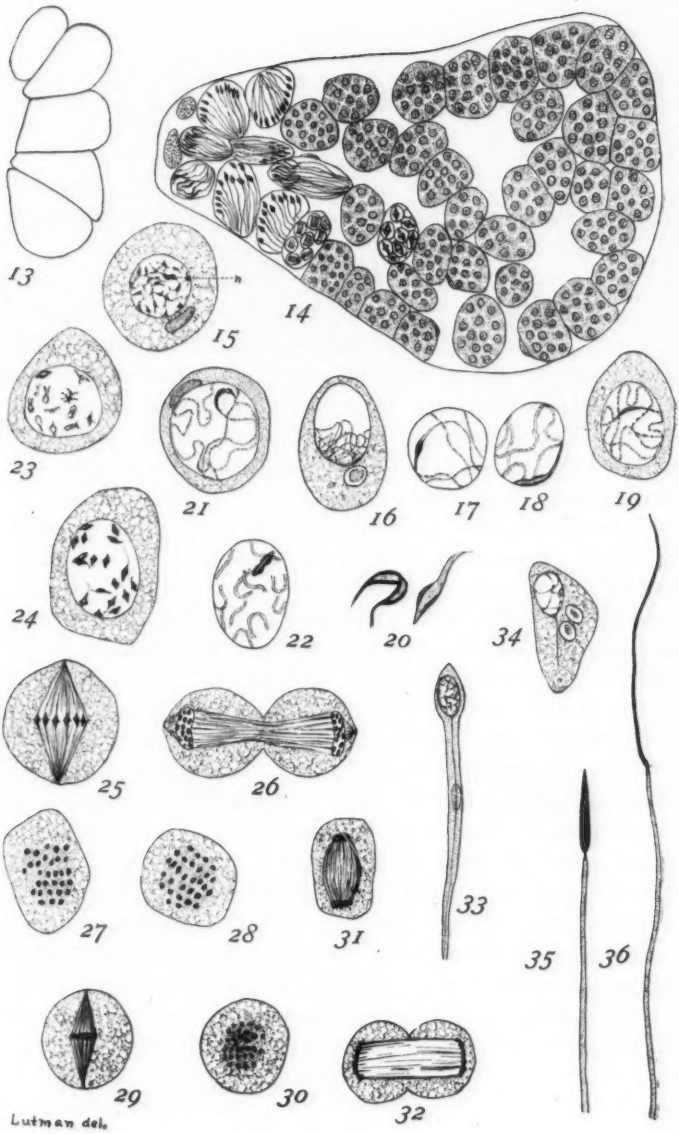
EXPLANATION OF PLATE II.

13. Longitudinal diagram of the entire mature testes.
14. Section of one of the follicles showing size, shape, and distribution of the cysts.
15. Formation of the spireme in a spermatocyte.
16. Synapsis.
17. Nucleus showing nucleolus on spireme.
18. Nucleus showing nucleolus on spireme.
19. Entire cell showing same.
20. Enlarged views of the nucleolus at this same stage showing the split.
21. Chromosomes formed by the transverse split.
22. Chromosomes lying free in the nucleus; also the split nucleolus.
23. Chromosomes forming the tetrads; nucleolus at *n*.
24. Tetrads.
25. Metaphase, first division.
26. Anaphase, first division.
27–28. Polar view of the equatorial plate.
29. Metaphase, second division.
30. Polar view, equatorial plate.
31–32. Anaphase, second division.
33–35. Transformation stages of the spermatid.
36. Sperm.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